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ÜBER DAS MENARCHEALTER IN FINNLAND

Sowie einige spezielle Beobachtungen über damit verbundene Faktoren

Akademische Abhandlung

VON

GRETA SIMELL

Almqvist & Wiksells Boktryckeri AB UPPSALA

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AUS DER KINDERKLINIK DER UNIVERSITÄT ZU TURKU
VORSTAND: PROF. DR. MED. TOIVO SALMI

ÜBER DAS MENARCHEALTER IN FINNLAND

SOWIE EINIGE SPEZIELLE BEOBACHTUNGEN ÜBER DAMIT VERBUNDENE FAKTOREN

AKADEMISCHE ABHANDLUNG
VON

GRETA SIMELL

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VORWORT

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Helsinki, im Februar 1952.

Die Verfasserin.

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I. Einleitung.

Schon bei den Völkern des Altertums ist die Bedeutung der Menarche bekannt gewesen. Ihrer Auffassung gemäss war die Frau nach dem Auftreten derselben geschlechtsreif. Seit den Zeiten des Talmuds hat man gewusst, dass die Mädchen früher reif werden als die Knaben und die in verschiedenen Verhältnissen lebenden Mädchen in verschiedenem Alter (Ellis). Bei den alten Ägyptern trat die Menarche früh ein, ungefähr im gleichen Alter wie in Europa im vorigen Jahrhundert, und im Griechenland des Altertums, in seiner Blütezeit, reiften die Frauen so früh, dass erst in den letzten Jahrzehnten, nachdem das Menarchealter bei uns abgenommen hat, diese Altersgrenze wieder nähergerückt ist (Mills). Seit Beginn des 19. Jahrhunderts hat man vor allem mit Hilfe von statistischen Untersuchungen versucht, die Faktoren zu klären, welche Einfluss auf das Menarchealter haben.

Die Menarche ist ein wichtiges Ereignis im Leben des Mädchens, und diese Erscheinung ist abhängig von sowohl inneren als auch äusseren Faktoren. In der Tätigkeit der innersekretorischen Drüsen geht aus irgendwelchen Gründen eine Umwälzung vor sich. Diese Zeitperiode dauert im allgemeinen ungefähr zwei Jahre, und man kann darin drei Hauptphasen unterscheiden. Biedl bezeichnet dieselben in chronologischer Reihenfolge als Pubeszenz, Adoleszenz und Maturität. Während der Praepubertät oder Pubeszenz sind das Längenwachstum des Mädchens und die kräftige Steigerung des Grundumsatzes sowie die ungewöhnliche Reizbarkeit des Nervensystems auffällig. In der Adoleszenz beginnt die Tätigkeit der Geschlechtsdrüsen, Hormone werden ins Blut ausgeschieden, und die sog. sekundären Geschlechtsmerkmale kommen allmählich zum Vorschein, während gleichzeitig die Gewichtszunahme das Längenwachstum überflügelt. In der Maturi-

tät sind die Keimdrüsen in den Vordergrund getreten, ihre Tätigkeit beginnt schon regelmässig zu werden, und das Wachstum ist nur noch geringes Nachwachsen. Auf die Reifung der Keimdrüsen wirken u.a. die Epiphyse, die Hypophyse, die Thyreoidea, die Nebennieren und die Thymusdrüse ein. Als ein Zeichen für die Tätigkeit der Geschlechtsdrüsen tritt dann die Menarche auf, was im allgemeinen am Ende des Pubertätsalters geschieht. Die Pubertätsperiode selbst ist dann noch nicht vorüber, sondern sie geht noch nach dem Auftreten der Menarche weiter, aber die erste Blutung kommt fast nie am Anfang der Entwicklungsperiode vor (Engle). Menstruationsähnliche Blutungen sind bisweilen auch bei kleinen Mädchen beobachtet worden, aber hier handelt es sich nicht um Menarche, denn der Unterschied zwischen Praecox- und Menarcheblutung liegt darin, dass die Praecoxblutung in einem ganz unfertigen Organismus auftritt (Schiötz).

Die Pubertätszeit ist auch vom Standpunkt des Paediaters wichtig, es steht ja nicht nur die Geschlechtsreife in Frage, sondern das Kind ist dann am empfindsamsten, in seinem Organismus geht eine gewaltige Entwicklung vor sich, und auch die geistigen Fähigkeiten reifen dann. Pratt sagt, es sei ein Wunder, dass diese Periode glücklich überwunden werde, wo alle Drüsen nach verschiedener Richtung ziehen. Was die Drüsen zur Tätigkeit bringt, hat man noch nicht zu entscheiden vermocht. Zahlreiche äusserliche Momente scheinen dabei viel Bedeutung zu haben, und gerade diese sind vom Standpunkt des Arztes aus wichtig, weil man sie wenigstens bis zu einem gewissen Grade auf vernunftgemässe Weise beeinflussen kann. Das frühe oder späte Auftreten der Menarche ist gewissermassen ein Massstab für das Kulturniveau eines Volkes, denn wenn bei einem Volke oder insbesondere bei einer Volksgruppe das Menarchealter bedeutend höher ist als bei den anderen, so hat man die Ursache in den äusseren Verhältnissen zu suchen (Schreiner). Schwankungen des Menarchealters sind zwischen den Individuen und Volksgruppen verschiedener Länder und auch des gleichen Landes beobachtet worden.

Im vorigen Jahrhundert herrschte allgemein die Auffassung, dass ein für das Menarchealter ausschlaggebender Faktor das Klima sei, oder besser gesagt der Breitengrad: Je näher des Äquators das Mädchen lebte. umso früher entwickelte es sich, je weiter

davon entfernt, umso langsamer (DUBOIS und PAJOT). FRAENKEL erklärt, dass das Menarchealter in Lappland 18 Jahre sei, um dann nach Süden zu immer mehr abzunehmen, bis es in Ägypten und Sierra Nevada 10 Jahre sei. ENGELMANN widersprach um die Jahrhundertwende der ausschlaggebenden Stellung des Breitengrads, welche Auffassung er so begründete: "Frühzeitige Menstruation im Alter von 9—10 Jahren in den Tropen und spätes Auftreten im Alter von 18—20 Jahren im hohen Norden ist eine Sage, die sich von Generation zu Generation von Buch auf Buch ohne jede wissenschaftliche Begründung fortpflanzt. Denn die frühzeitige Pubertät in den tropischen Gegenden ist so wie deren spätes Auftreten in den arktischen Regionen nicht als Regel anzusehen. Das Negermädchen in Somaliland entwickelt sich mit 16 Jahren also ebenso spät wie die Lappländerin, und Samojedinnen und Eskimoweiber können ebenso wie die Hindufrau aus Indien schon mit zwölf Jahren Mutter werden. Die Pubertät kann am Pol ebenso früh auftreten wie dies gewöhnlich am Äquator angenommen wird und am Äquator so spät, wie man es für die Polbewohnerinnen annimmt". Nach ENGELMANN haben zahlreiche Forscher bewiesen, dass das Pubertätsalter nicht durch den Abstand vom Äquator bestimmt wird (YAMASAKI, GOULD und GOULD).

Der Norweger SKERJL glaubte aus seinem Material feststellen zu können, dass nicht der Breitengrad, sondern der Längengrad, die Windverhältnisse sowie die Höhe vom Meeresspiegel des Wohnorts die Faktoren seien, welche zum grossen Teil das Menarchealter bestimmen.

Auch die Untersuchungen von MILLS betrafen das Klima und nach seiner Theorie ist die Reifung davon abhängig, wie leicht oder schwer das Schwitzen vor sich geht. Leichtes Schwitzen fördert das Wachstum und die Entwicklung, und deshalb werden die in gemässigtem, windigem Klima lebenden Mädchen kräftiger und menstruieren früher als die in der tropischen Feuchtigkeit lebenden Frauen. Erwähnt sei noch, dass MILLS in der Reifungswie auch in der Wachstumserscheinung wogenförmige Bewegung wahrnahm, welche sich im Wechsel der Zeitalter den Schwankungen des Klimas angleicht. Im Mittelalter war die Temperatur niedriger, was den Prozess verlangsamte, danach förderte die erhöhte Temperatur denselben, und nun gehen wir wieder einer

kühleren und langsamer entwickelnden Zeit entgegen. Höwe bringt eine etwas eigenartige Theorie über den Einfluss des Klimas auf die Menarche vor. Hiernach würde durch die Zimmerheizung das Klima der gemässigten Zonen erzeugt, desgleichen durch die Kleidung.

Die frühere Reife der Städter im Vergleich zur Landbevölkerung ist im allgemeinen dem lebhaften, nervenreizenden Lebenstakt der Stadt zugeschrieben worden. Die Menarche als eine Rasseneigenschaft ist schon im vorigen Jahrhundert festgestellt worden, insbesondere durch Vergleiche zwischen dem Menarchealter der Juden und dem entsprechenden Alter bei ihrem Wirtsvolke. Desgleichen hat man auch den förderlichen Einfluss der günstigen äusseren Verhältnisse wahrgenommen, obwohl Fanconi der Ansicht ist, dass die Pubertät umso früher käme, je primitiver die in Frage stehende Rasse ist. Die Art der Arbeit, die Nahrung, die Vitamine, die sozialen Verhältnisse, die Erblichkeit, die ultraviolette Strahlung, alle diese Umstände dürften ihren Anteil an der Reifung der Frau haben. Alle diese verschiedenen Beobachtungen haben zu der Auffassung geführt, dass keiner von diesen Momenten allein das Menarchealter zu bestimmen vermag, sondern dass es die Summe zahlreicher sowohl innerlicher als auch äusserlicher Faktoren ist.

Auch in *Finnland* ist seit dem vorigen Jahrhundert das Menarchealter untersucht worden. Im Jahre 1882 berechnete Heinricius auf Grund seines in der Gebärabteilung der Universitätsklinik zu Helsinki gesammelten Materials, welches in der Hauptsache Frauen aus sozial ungünstigen Schichten enthielt, das Menarchealter auf 15,82 Jahre. Zwischen den in der Stadt und den auf dem Lande geborenen Frauen stellte er hier keinen Unterschied fest, ebensowenig zwischen den finnisch- und schwedischsprechenden. Er erachtete die Rasse, die klimatischen Momente sowie die Vermögensverhältnisse und die Lebensweise als wichtige Faktoren für das Menarchealter. v. Heideken hat Erstgebärende untersucht, deren Menarchealter er auf 15,99 Jahre schätzte. Engström sammelte sein Material aus den vermögenderen Volksklassen in seiner Privatpraxis in Helsinki. Er erhielt ein niedrigeres Menarchealter, nämlich 15,07 Jahre, was seines Erachtens gerade auf die Vermögensverhältnisse zurückzuführen war. Nyström, der in der Hauptsache Ovarialtumoren untersuchte,

bestimmte auch das Menarchealter, welches bei den von ihm untersuchten Patientinnen 15,89 Jahre war. WARÉN sammelte ein gemischtes Material aus beiden Sprachengruppen, und aus allen sozialen Klassen. Der von ihm berechnete Wert war 15,14 Jahre. Er bestimmte auch die obere und untere Grenze des Menarchealters, wobei er für die erstere 17 Jahre und für die letztere 12 Jahre erhielt.

Alle die obengenannten Autoren haben aus ihren Fällen nur die mathematischen Mittelwerte berechnet. MALMIO dahingegen gebrauchte im Jahre 1919 als Erster bei uns in Finnland die moderne statistisch-mathematische Berechnungsweise. MALMIOS Material war auf Grund von Krankenberichten zusammengestellt. Ausser 19395 Fällen aus der Universitätsklinik, wo die Patientinnen in erster Linie aus den minderbemittelten Arbeiterklassen stammten, sammelte er in einem helsinkier Privatkrankenhaus noch die Angaben über 1071 von den allerreichsten unter den höheren sozialen Schichten, sog. feineren Patientinnen. Um eine Zwischenstufe zu bekommen, nahm er in sein Material noch aus der Diakonissenanstalt Patientinnen aus der Mittelklasse auf. Unter den zur Arbeiterklasse gehörenden finnischen Frauen aus beiden Sprachengruppen erhielt er ein mittleres Menarchealter von 16,09 ± 0,05 Jahren. Die zur schwedischsprechenden Arbeiterbevölkerung zählenden Frauen menstruierten nach MALMIOS Berechnung ½ Jahr früher als die finnischsprechenden. Der entsprechende Wert des Materials aus dem Privatkrankenhaus war ½ Jahr niedriger als bei den schwedischsprechenden Arbeiterfrauen, und das nach dem Material der Diakonissenanstalt berechnete Menarchealter lag zwischen den beiden erstgenannten, obwohl der Unterschied zum Menarchealter der Arbeiterfrauen nicht signifikant war. Als Differenz zwischen dem Pubertätsalter der Arbeiterfrauen und der besser situierten erhielt er 1 Jahr. Er wies auf die Vererbung des frühen Menarchealters hin und verband damit das Fortdauern anderer sozialer Eigenschaften in der Familie. Die Bedeutung der Umgebung für das Menarchealter war nach MALMIOS Ansicht sehr übertrieben worden.

Im Zusammenhang mit seinen Strumaforschungen hat KAARTINEN aus seinem Material auch das Menarchealter berechnet, indem er die Strumafälle mit normalen Fällen verglich. Bei den

Strumaträgern war der Wert niedriger als bei den strumafreien Patientinnen, 14,9 Jahre und 15,3 Jahre. A. Vuori hat in seiner Arbeit über die Heredität der Blutgruppen ebenfalls das Menarchealter seines Materials bestimmt, welches aus Schülerinnen der Gemeinschule und des Seminars zu Hämeenlinna bestand. Vuori wollte untersuchen, ob zwischen den Blutgruppen und dem Menarchealter eine Korrelation besteht, und das Resultat war negativ. Sowohl das aus der Gemeinschule als das aus dem Seminar stammende Material war klein, und das Menarchealter war bei dem ersteren 13,9 und bei dem letzteren 14,8 Jahre.

E. Pelkonen berichtet in seiner Untersuchung über den Volksglauben, dass die Menarche als ein Zeichen dafür aufgefasst wurde, dass das Mädchen das Reifealter erreicht hatte und befruchtungsfähig war. Desgleichen herrschte die Ansicht, dass die Menarche gewöhnlich zwischen 14 und 16 Jahren eintritt, und wenn im Alter von 16 Jahren noch keine Blutung vorgekommen war, mussten Massnahmen ergriffen werden. Dahingegen glaubte das Volk in den abgelegenen, rückständigen und armen Gegenden, dass noch im Alter von 20 Jahren die Menstruation ganz normal beginnen könne. Nach der volkstümlichen Auffassung hatten die Konstitution und das Temperament grossen Einfluss auf den Beginn der Blutung. Man glaubte, dass bei den blutvollen, wohlbeleibten, lebhaften Mädchen die Menstruation im Alter von 11—12 Jahren normal beginnen könne, während sie bei den mageren, stillen und kränklichen Frauen verspätet eintreten kann, ohne dass Ursache zu Besorgnis vorläge. Vara bestimmte das Menarchealter in seinem 11406 Fälle umfassenden Material auf 14,98 ± 0,02 Jahre (14,977 ± 0,0222 Jahre). Das Material stammte aus der Frauenklinik zu Helsinki und war gesammelt in der Zehnjahresperiode 1927—1936. Nach Ansicht der Verfassers wirken sowohl endo- als auch exogene Faktoren auf das Menarchealter. In einer anderen Veröffentlichung hat er berechnet, dass die Menarche bei den alten Erstgebärenden im Alter von 15,41 ± 0,05 Jahren eingetreten war, und Vehaskari berechnete bei Primärsterilitätsfällen den gleichen Wert auf 14,7 ± 0,1 Jahre. E. Aschans Arbeit aus dem Jahre 1950 betraf die Neigung der Hypoplastikerinnen zu Abort, und er bestimmte im Zusammenhang damit auch das Menarchealter, welches hoch war, 17,7 Jahre. Bei

der den Finnen verwandten Bevölkerung von Aunus berechnete Reenkola im Jahre 1945 auf Grund von 266 Fällen das Menarchealter auf 15,8 Jahre. Interessant ist, dass bei diesem Stamme kein Sinken des Menarchealters im Laufe einer Generation stattgefunden hatte. Das Material von Reenkola umfasst nämlich zwei Generationen. Jääməri hat auch bei den Frauen des mit den Finnen verwandten onega-wepsischen Stammes nach ihrem Pubertätsalter gefragt, welches 13—14 Jahre war.

Alle die bisher erwähnten Autoren, welche das Menarchealter untersucht haben, sind Ärzte gewesen, aber auch die Pädagogen haben sich für diese Angelegenheit interessiert. Salo hat seinem pädagogischen Werk auch ein Kapitel über die Menarche angefügt. Um das Menarchealter der finnischen Mädchen genau zu bestimmen, sammelte Salo aus den Frauenseminaren ein Material, in welchem er nur diejenigen 542 Fälle berücksichtigte, bei welchen die Menarche auf den Tag angegeben wurde. Das hiernach berechnete Alter war 14,22 Jahre. Salo schloss sich Malmios Ansicht über die parallele Beschleunigung der geistigen und körperlichen Entwicklung nicht an, sondern er vermutete, dass längere Reifezeit in jeder Beziehung besseres Resultat bedeute.

Am Anfang des 20. Jahrhunderts erweckte die Tatsache, dass bei den jüngeren Generationen das Menarchealter niedriger war als bei den älteren, Interesse. Da ausserdem festgestellt worden war, dass der Unterschied zwischen den verschiedenen Menschengruppen, z.B. zwischen Land- und Stadtbevölkerung, zwischen den sog. höheren und niedrigeren Klassen, den vermögenderen und unbemittelten, sich immer mehr ausglich, wurde angenommen, dass dies wenigstens zum Teil eine Folge der um die Jahrhundertwende und danach geschehenen gesellschaftlichen und sozialen Veränderungen sei.

Finnlands abgelegene Lage, seine Grösse und dünne Besiedelung haben dazu geführt, dass noch am Ende des 19. Jahrhunderts ein deutlicher Unterschied zwischen der Stadt- und der Landbevölkerung bestand. Die um diese Zeit gebauten Eisenbahnen und das Landstrassennetz, welche in diesem Jahrhundert und insbesondere nach der Autonomie unseres Staates schnell zugenommen haben, verbinden schon die verschiedenen Landesteile miteinander. Im Jahre 1921 trat der allgemeine Schul-

zwang in Kraft, und infolgedessen kommt jedes Kind mit Fremden in Berührung, bekommt neue Anregungen und lernt neue Sitten. Früher war die Kinderpflege und die Gesundheitspflege mangelhaft, die Wohnverhältnisse schlechter als heutzutage, und die hygienischen Verhältnisse liessen viel zu wünschen übrig. So richteten z.B. die Seuchen grosse Verheerung an, die Tuberkulose war allgemein verbreitet, und die Sterblichkeit der Kleinkinder war erschreckend gross. Infolge der mangelhaften Kinderpflege war die Rachitis auch in schwerer Form sehr häufig. Der im Jahre 1920 gegründete General Mannerheims Kinderschutzverbund arbeitet für die Förderung der körperlichen und geistigen Gesundheit der aufwachsenden Generation, und ihm ist es zum grossen Teil zu verdanken, dass die Sterblichkeit der Kleinkinder gesunken und die Jugend gesunder als früher ist, und dass die Mädchen, nachdem sie zu Frauen gereift sind, eine bessere Stellung haben. Die Abhängigkeit des Menarchealters von der Beschaffenheit der Nahrung ist eine bekannte Tatsache. Früher war besonders in den Landgegenden die Nahrung einseitig und die Bedeutung der rohen Nahrungsmittel unbekannt. Wenn Mangel und Hungersnot die Bevölkerung bedrückten, hatten insbesondere die Unbemittelten darunter zu leiden, wass Steigerung des Menarchealters zur Folge hatte. In den letzten Jahrzehnten ist die Nahrung besser geworden, und der Wert der verschiedenen Nahrungsmittel sowie z.B. die wichtige Bedeutung der Vitamine ist beim Volke bekannt.

Die Arbeiterbewegung, welche sich im vorigen Jahrhundert nach Finnland ausbreitete, hat in diesem Jahrhundert gewaltiges Ausmass angenommen, indem sie in erster Linie die minderbemittelten Volksschichten beeinflusst. Malmio u.a. erachtet das Sinken des Menarchealters zum grossen Teil für eine Folge der Arbeiterbewegung. Auch wirtschaftlich hat im 20. Jahrhundert ein Aufstieg stattgefunden. Früher musste das Landmädchen schon als Kind an der Feldarbeit teilnehmen, die jüngeren Geschwister versorgen und unter dem Zwang der Not sogar noch im Wachstumsalter selbst seinen Unterhalt verdienen. Heutzutage ist die Jugend durch Gesetze geschützt. Auch der Rundfunk trägt dazu bei, den Unterschied zwischen den Landgegenden und der Stadt auszugleichen. Mit Hilfe von Sport und Turnen entwickelt

sich der körperliche Zustand der jungen Menschen, und das gesunde, frische Mädchen ist heutzutage viel besser für die Reife entwickelt als das ungeübte Mädchen von früher. Die Literatur kann ebenfalls reifenden Einfluss haben, aber wenigstens ein Teil davon erweckt allzu früh die sexuelle Phantasie der jungen Menschen. Das gleiche dürfte sich wohl von der heutigen Tanzmusik und den Filmen sagen lassen.

Die Kriegsjahre 1939—1944 sind sowohl geistig als auch körperlich für das ganze finnische Volk schwer gewesen. Das Jahr des Zwischenfriedens war so anormal, dass auch dies als Kriegszeit gerechnet werden kann. Da die Stadtbevölkerung zum grossen Teil auf das Land geflohen war, gestaltete sich auch das Leben der Landbevölkerung schwierig und beengt. Die Lebensmittellage war schlecht. Die Rationen waren klein und insbesondere in der Stadt nicht immer zu bekommen. Bezüglich des Kaloriengehalts befriedigte die Nahrung knapp den Bedarf des Organismus, obwohl für den Teil der Kinder und Jugendlichen versucht wurde, durch zusätzliche Rationen schädliche Wirkungen abzuwehren. Vitaminmangel, insbesondere C-Vitaminmangel, wurde festgestellt. Am schwersten hatten unter den Folgen des Krieges die Karelier zu leiden, welche zweimal ihre Heimat verlassen mussten.

II. Bergründung der Arbeit und Fragestellung.

Im laufenden Jahrhundert haben WARÉN sowie MALMIO ihre Untersuchungen über das Menarchealter veröffentlicht. MALMIOS Material enthält Fälle aus der Gebäranstalt und dem gynäkologischen Krankenhaus bis zum Jahre 1917. Danach ist diese Sache in unserem Lande nicht mehr einheitlich untersucht worden. In Finnland hat jedoch, wie schon oben gezeigt wurde, in diesem Jahrhundert die Entwicklung auf den verschiedenen Gebieten grosse Veränderungen der Verhältnisse mit sich gebracht. Die finnische Bewegung ist stark gewesen, der Wohnort bestimmt nicht mehr in so grossem Masse wie früher das äussere und innere Leben, die Klassengrenzen sind im Verschwinden begriffen, und in den Vermögensverhältnissen trifft man nicht mehr so grosse Unterschiede wie im vorigen Jahrhundert. Die Kriegsjahre sind für unser Volk eine schwere Prüfung gewesen, und die Umsiedelungen haben nach der Abgabe von Landesteilen grosses Ausmass angenommen. Wenn man all dies berücksichtigt, und wenn man aus der heutigen Literatur über die Menarche entnimmt, welche grosse Rolle jeder von diesen Faktoren für das Menarchealter spielt, ist es meines Erachtens vollauf berechtigt, diese Fragen bei uns in Finnland jetzt wieder aufzunehmen.

Bis jetzt hat das Menarchealter in erster Linie die Gynäkologen interessiert, aber das in diesem Jahrhundert wahrzunehmende Sinken des Menarchealters veranlasst auch den Paediater, sicht mit dieser Angelegenheit zu beschäftigen. Der eigentlichen Menarche geht ja ein ungefähr zwei Jahre dauerndes schweres Entwicklungsstadium, die Pubertätszeit voraus, während welcher gerade der Kinderarzt dazu berufen ist, dem Mädchen in seinen sowohl körperlichen als auch geistigen Schwierigkeiten zu helfen.

Die meisten früheren Autoren haben ihre Materialien auf Grund der Krankenberichte aus Entbindungsanstalten und gynäkologischen Krankenhäusern gesammelt, da aber in diesen Krankenblättern das Menarchealter bestenfalls nur auf ein Jahr genau angegeben ist, bin ich nicht so verfahren. Da ferner anzunehmen ist, dass die soeben ihre Entbindung erwartende Mutter sich nicht in die an sie gerichteten, ihres Erachtens so unwichtigen Fragen zu vertiefen vermag, sind die Angaben der Krankenberichte für die Berechnung des genauen Menarchealters unzuverlässig. Ausserdem gelangt in unserem Lande ein grosser Teil der Frauen überhaupt nie in ein Frauenkrankenhaus, weder in die gynäkologische noch in die Entbindungsabteilung, weshalb ich, um einen Durchschnitt vom Menarchealter der Frauen, auch der unverheirateten, in unserem Lande zu erhalten, mein Material anderwärts gesammelt habe.

Mit meiner Arbeit möchte ich folgendes klären:

Welches ist jetzt das Menarchealter in Finnland?
Hat der Krieg Einfluss auf das Menarchealter gehabt?
Einfluss der Sprach- und Rassenverteilung auf die Menarche sowie dieses Alter bei unseren verschiedenen Stämmen.
Hat der Beruf Einfluss auf das Menarchealter?
Welchen Einfluss haben der Wohnort und die zwangsweisen Umsiedelungen auf das Menarchealter?
Einige spezielle, mit der Menarche verbundene Beobachtungen, wie das Verhältnis der Menarche zur Regelmässigkeit der Menstruation und den Menstruationsbeschwerden, den Leistungen in der Schule, sowie das Verhalten der Mädchen, welche schmerzhafte Menstruation haben, zu den Leibesübungen.
Verteilung der Menarche auf die verschiedenen Kalendermonate.

III. Das Material und die Untersuchungsmethoden.

Das Material, anhand von welchem ich das Menarchealter berechnet habe, umfasst 3057 Fälle und wurde in der Zeit vom Mai 1949 bis Ende des Jahres 1950 gesammelt. Das Material stammt aus verschiedenen Gegenden unseres Landes und enthält Mädchen aus allen Schichten des finnischen Volkes, aus beiden Sprachgruppen, sowohl finnisch- als auch schwedischsprechende, sowohl auf dem Lande als in der Stadt wohnende, zwischen den Jahren 1923 und 1933 geborene Mädchen. Das ganze Material umfasst 5741 Fälle, aber die nach dem Jahre 1933 geborenen habe ich bei der Berechnung des durchschnittlichen Menarchealters nicht mitgenommen, weil sie zu einer zu jungen Altersgruppe gehören. Bei den anderen Berechnungen, wie z.B. das Auftreten der Menarche in den verschiedenen Kalendermonaten, habe ich allerdings auch diesen Teil meines Materials benutzt. Für meinen Zweck benutzte ich Karten, in welchen ausser dem Geburtsdatum und Geburtsort auch der Wohnort, der Beruf des Vormundes, die Körperlänge, das Gewicht, die früheren Krankheiten und der Zeitpunkt der Menarche sowie wann die Menstruation regelmässig geworden war, verzeichnet wurden. Ausser diesen Angelegenheiten wurde nach den Menstruationsschmerzen gefragt sowie ferner, ob das betreffende Mädchen während der Menstruation nicht an den Leibesübungen teilnimmt. Die Antworten erhielt ich im allgemeinen leicht, das Menarchealter wussten die meisten auf den Monat genau, ein Teil auch auf den Tag.

Bei meinen Berechnungen habe ich eine Gruppierung angewandt, wonach z.B. diejenigen, welche im Alter von 13,6 Jahren bis 14,5 Jahren ihre Menarche bekommen hatten, zur Altersgruppe 14 Jahre zählten. Mein Material habe ich zum grössten Teil per-

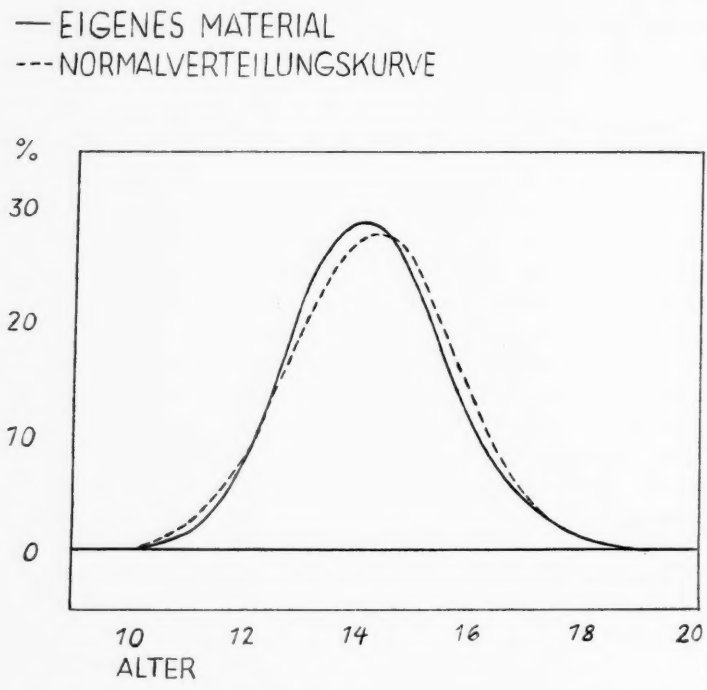
sönlich gesammelt. In meiner Privatpraxis habe ich von Patientinnen und ihren Müttern aus beiden Sprachgruppen und aus allen sozialen Schichten sowohl aus der Stadt- als auch aus der Landbevölkerung die erforderlichen Angaben in über 600 Fällen bekommen. Im Sommer 1949 war ich im Grosslager der Pfadfindermädchen als Ärztin tätig. Alle Teilnehmerinnen wurden untersucht, und bezüglich der nach dem Jahre 1923 Geborenen wurden meine Karten ausgefüllt. Das Lager war zweisprachig, so dass sowohl die finnisch- als auch die schwedischsprechende Bevölkerung vertreten war, und im ganzen erhielt ich nahezu 1000 Fälle aus verschiedenen Gegenden Finnlands. Einige Kollegen sowie auch Gesundheitsschwestern in verschiedenen Landesteilen sind mir beim Sammeln des Materials behilflich gewesen, indem sie meine Karten über ihre eigenen Patientinnen ausfüllten. In den Lehranstalten, in welchen ich nicht persönlich Angaben sammelte, haben die Turnlehrer und Schulschwestern meinen Instruktionen gemäss die Karten ausgefüllt. Mein Material wurde sowohl in den Fortbildungsklassen der Volksschulen, in Fachschulen, Mittelschulen und verschiedenen landwirtschaftlichen Lehranstalten sowie Volkshochschulen, in ärztlicher Praxis und verschiedenen Mutterschaftsberatungsstellen sowie bei Privatpersonen gesammelt. Das Gewicht und die Länge wurde bei den Schülerinnen aus den Gesundheitskarten erhalten, in der Privatpraxis und im Lager wurden sie gemessen. Bei der Einteilung nach dem Wohnort wurden die in den Marktflecken wohnenden Mädchen als Städterinnen angesehen.

Den Einfluss der Menarche auf die geistigen Leistungen habe ich nur bei den Schülerinnen der höheren Schulen untersucht, indem ich die Mittelwerte der wisschenschaftlichen Fächer auf ¼ Jahr genau berechnete. Da viele Mädchen während der Kriegszeit die Stadt verlassen und deshalb ihren Schulbesuch unterbrechen mussten, schmolz mein Material ziemlich zusammen, desgleichen wurde es durch Repitieren und schwere Krankheiten während der Schulzeit verkleinert, so dass es schliesslich nur 139 Fälle umfasst. Die Jahresklassen 1923 und danach Geborenen wählte ich aus, um die Einwirkung des Krieges auf die Entwicklung der Mädchen untersuchen zu können, wobei ich besondere Aufmerksamkeit den von den abgegebenen Gebieten umgesiedelten

Mädchen zuwandte, da der Krieg insbesondere für sie sehr schwer gewesen ist.

Da die Gruppierung eines so grossen Materials nach den verschiedenen Prinzipien mit der Hand schwierig gewesen wäre, liess ich entsprechende Lochkarten anfertigen. Im ganzen sind 15 verschiedene Gruppierungen ausgeführt worden und innerhalb einer jeden Gruppe ausserdem noch Einteilung nach dem Alter. Die von den in den Jahren 1923—1933 Geborenen gezeichnete Verteilungskurve liegt der Normalverteilung sehr nahe. Figur Nr. 1.

Figur Nr. 1.



In meinen Berechnungen habe ich das arithmetische Mittel *M* nach der Formel $M = \frac{1}{n} \Sigma n_i x_i$ bestimmt, worin n die Anzahl der Fälle in dem zu untersuchenden Material ist, n_i die Anzahl

in der i. Altersklasse und x_i der Mittelwert dieser Klasse. Σ ist das Zeichen für die Summe. Desgleichen habe ich die Formel

$$M = M_o + \frac{1}{n} \Sigma\, n_i\, (x_i - M_o)$$

angewandt, worin M_o der provisorische Mittelwert ist. Zur Berechnung der Streuung σ habe ich die Formel

$$\sigma^2 = \frac{1}{n} \Sigma n_i\, (x_i - M_o)^2 - (M - M_o)^2$$

benutzt. Der mittlere Fehler des arithmetischen Mittels wurde berechnet aus der Formel: $\varepsilon\,(M) = \frac{\sigma}{\sqrt{n-1}}$; den mittleren Fehler der Differenz von zwei von einander unabhängigen arithmetischen Mitteln M_1 und M_2 erhielt ich aus der Formel

$$\varepsilon\,(M_1 - M_2) = \sqrt{\varepsilon^2\,(M_1) + \varepsilon^2\,(M_2)}$$

Als signifikant ist die Differenz zwischen zwei Werten angesehen worden, wenn sie grösser war als $2 \times \varepsilon$. Zur Bestimmung des mittleren Fehlers eines Prozentes wurde die Formel

$$\varepsilon_{(p)} = \sqrt{\frac{p \times q}{n}} \text{ benutzt,}$$

worin p = Prozent ist und q = 100 — p.

Insbesondere bei den Berechnungen, welche das Auftreten der Menarche in den verschiedenen Kalendermonaten betreffen, habe ich für die Fehlerberechnungen RITALAS Tabellen angewandt.

Im Folgenden werden die Resultate in der Form $M \pm \varepsilon\,(M)$ wiedergegeben.

IV. Eigene Untersuchungen.

1. Das Menarchealter in Finnland.

Auf Grund von 3057 Fällen meines Materials, welche alle in den Jahren 1923—1933 geborene finnische Mädchen waren, berechnete ich für das Menarchealter im Jahre 1950 in Finnland *14,25 ± 0,03 Jahre*. Die Extremfälle in meinem Material waren 9 Jahre 10 Monate und 22 Jahre 2 Monate. Meistens tritt die Menarche in der Zeit zwischen 11,6 Jahren und 17,5 Jahren ein. In den verschiedenen Jahresklassen hatten die im Jahre 1925 geborenen Mädchen in meinem Material das höchste Menarchealter, 14,57 ± 0,14 Jahre, und das niedrigste entsprechend die im Jahre 1930 geborenen, 14,04 ± 0,02 Jahre.

Die Zeit nach dem Auftreten der ersten Menstruation ist in der Entwicklung des jungen Mädchens sehr bedeutungsvoll, denn erst danach beginnt ein wichtiger Teil des Organismus' seine normale Tätigkeit. Ritala z.B. berechnet das Fertilitätsalter der Frau vom Auftreten der ersten Menstruation an.

Insbesondere in unserem Jahrhundert ist festgestellt worden, dass das Menarchealter in hohem Masse vom Geburtsjahr abhängig ist, denn je später das Mädchen geboren ist, in umso jüngerem Alter tritt bei ihm durchschnittlich die Menarche auf.

Nach der Jahrhundertwende wurde in den Schulen beobachtet, dass die Entwicklung der Kinder rascher wurde. Sie waren im Vergleich zu den gleichaltrigen Kindern aus dem vorigen Jahrhundert grösser, und die Menarche trat bei den Mädchen früher ein. Im Jahre 1914 sprach Helms in Dänemark als Erster die Vermutung aus, dass das Menarchealter im Laufe der Zeit gesunken sei (Malmio). Die meisten Autoren, welche diese Accelerations-Erscheinung der Menarche untersuchten, verglichen das

entsprechende Alter der Mütter und ihrer Töchter miteinander, auf welche Weise sie ihr Material aus den gleichen äusseren Verhältnissen erhielten.

Nach Bolk trat in Holland im Jahre 1926 bei der jüngeren Generation die Menarche ungefähr ein Jahr früher auf als bei der älteren Generation. Charakteristisch für das Jüngerwerden war es, dass die Anzahl der früher, ca. im Alter von 10 Jahren reifenden Mädchen zunahm. Ähnlich war die Situation auch im Jahre 1926 in Deutschland (Stein), und noch grösser war der Unterschied dort im Jahre 1934, nach Petri 18,4 Monate. Nach Mirow wiederum war das Menarchealter seit dem Jahre 1870 um nahezu 2 Jahre gesunken. Diese Zahlen stammen aus dem Jahre 1943. In Schweden hat Samuelsson berechnet, dass während der letzten 80—100 Jahren das Menarchealter um 2—2½ Jahre gesunken ist. Nach den von Lennér im Jahre 1944 veröffentlichten Mitteilungen ist in Schweden das Menarchealter während der letzten 50 Jahre um durchschnittlich 10 Tage pro Jahr gesunken. Wilson und Sutherland in England haben diese Verjüngungserscheinung im Jahre 1949 nicht festgestellt, aber ihr Material war auch viel zu klein.

Malmio hat ebenfalls das Sinken des Menarchealters in Finnland untersucht. Aus seinem eigenen und *v. Heidekens* Erstgebärenden-Material berechnete er, dass bei den in den Jahren 1879—1887 Beobachteten die Menarche durchschnittlich im Alter von 16,65 ± 0,06 Jahren aufgetreten war, und bei den in den Jahren 1908—1915 Beobachteten im Alter von 16,02 ± 0,03 Jahren. Demnach trat die Menarche bei den in den Jahren 1908—1915 Beobachteten Mädchen 0,63 ± 0,07 Jahre früher ein als bei den ersteren.

Mein eigenes Material stammt aus einer sehr kurzen Zeitperiode; es umfasst die im Jahre 1923 und danach Geborenen. Für diesen Teil meiner Berechnungen nahm ich nur 8 Jahresklassen mit, denn ich schied auch die im Jahre 1931 Geborenen aus, um zu vermeiden, dass eine zu junge Generation mit dazukäme. Wenn als Trend eine Parabel zweiten Grades benutzt worden wäre, so wäre der niedrigste Wert der Kurve auf die in den Jahren 1928—1929 Geborenen gekommen, um dann wieder zu steigen, aber dies hätte nicht der Wirklichkeit entsprochen. Die

Anwendung des geraden Trends war natürlich auch nicht richtig, weil man hier von Jahr zu Jahr zu einem immer jüngeren Alter kommt. Als Formel des geraden Trends ergab sich:

$$y = -0{,}155\,X + 3{,}2,$$

worin X = Geburtsjahr und y = Menarchealter ist.

Im Jahre 1882 berechnete HEINRICIUS als Mittelwert des Menarchealters 15,82 Jahre. MALMIO, welcher sich darauf berief, dass das Menarchealter, wenn es auf ein Jahr genau mitgeteilt wird, im allgemeinen um ein halbes Jahr zu früh angegeben wird, korrigierte den Wert auf 16,62 Jahre. MALMIOS eigener Wert war $15{,}95 \pm 0{,}01$ Jahre. Das von VARA im Jahre 1943 berechnete Menarchealter bei Erstgebärenden war $14{,}98 \pm 0{,}02$ Jahre und das Resultat aus meinem Material wiederum war $14{,}25 \pm 0{,}03$ Jahre.

Das Menarchealter in verschiedenen Jahren in Finnland:

Jahr	Autor	Menarchealter	
1882	HEINRICIUS	16,62	Jahre
1919	MALMIO	$15{,}95 \pm 0{,}01$	„
1943	VARA	$14{,}98 \pm 0{,}02$	„
1950	SIMELL	$14{,}25 \pm 0{,}03$	„

Seit dem Jahre 1882 ist das Menarchealter in Finnland um 2,37 Jahre und seit dem Jahre 1919 um $1{,}70 \pm 0{,}03$ Jahre gesunken.

Das Jüngerwerden des Menarchealters zeigt sich am deutlichsten im Vorkommen der frühen Menarche. In Deutschland nahm die Anzahl der unter 12 Jahren reifenden Mädchen im 20. Jahrhundert von 13 % auf 18 % zu. Die Anzahl derjenigen Mädchen wiederum, bei denen die Menarche spät, im Alter von über 16 Jahren auftrat, sank von 26 % auf 5 % (NEVINNY-STICKEL). Bei uns lässt sich auf Grund der Tabelle 1 keine Zunahme derjenigen, bei denen die erste Menstruation im Alter von unter 11,6 Jahren vorkam, feststellen, dahingegen scheint die Anzahl der im Alter von über 17,5 Jahren reifenden Mädchen abzunehmen.

Als Ursache für diese auf der ganzen Welt wahrzunehmende Verminderung des Menarchealters wird die allgemeine Entwicklung auf wirtschaftlichem, hygienischem und allgemeinkulturellem Gebiete angesehen. Der Anteil der Arbeiterbewegung ist beträchtlich (Malmio, Lennér), aber auch die Gesundheitspflege, welche den Mädchen grössere Möglichkeiten bietet, sich ungeschädigt zur Frau zu entwickeln, wirkt sehr auf die Entwicklung ein, ebenso wie der ganze schnellere Lebensrhythmus. Die Folge ist jedenfalls, dass das Kindesalter immer kürzer wird, sofern nicht, wie z.B. Mills vermutet, das Menarchealter in den nächsten Jahren wieder zu steigen beginnt.

Berechnet auf Grund eines Materials von 3057 Fällen war das Menarchealter im Jahre 1950 in Finnland 14,25 ± 0,03 Jahre.

Seit dem Jahre 1882 ist das Menarchealter in Finnland um 2,37 Jahre gesunken und seit dem Jahre 1919 um 1,70 ± 0,03 Jahre.

2. Der Einfluss des Krieges auf das Menarchealter.

Die Kriege, die wir durchzustehen hatten, sind totale Kriege gewesen. Die psychischen Belastungen bedrückten alle, der Mangel an Schlaf, die Umsiedelungen und die mangelhafte Nahrung erschwerten das Leben. Auch die Zeit des ungefähr ein Jahr lang dauernden Zwischenfriedens war insofern anormal, als eine eigentliche Friedenszeit nicht wiederhergestellt werden konnte. Der Nahrungsmangel hatte auf die stillenden Mütter so grossen Einfluss, dass in der Stillfrequenz und — dauer deutliches Sinken wahrzunehmen war, wie Salmi gezeigt hat. Unter diesen Umständen könnte man wohl annehmen, dass die wachsenden, empfindlichen, im Pubertätsalter stehenden Mädchen auch so viel gelitten hätten, dass ihre Entwicklung sich verzögert hätte. Schon allein die mangelhafte Nahrung hätte das Reifen beeinträchtigen können, denn durch den Hunger können die endokrinen Drüsen so schwer geschädigt werden, dass ihre Tätigkeit später gestört ist (Stefko). So schlecht ist unsere Lebensmittellage jedoch während der ganzen Kriegszeit niemals gewesen. Ausgesprochenen Hunger

brauchte wohl kaum jemand zu leiden, aber auch ein geringerer Hungerzustand genügt, um ungünstig wirken zu können (HÄBERLIN). Auch geringerer Nahrungsmangel kann sogar so tief wirken, dass die Zusammensetzung der Muttermilch sich wesentlich ändert. Dies hat bei uns SALMI durch seine Untersuchungen aus den Jahren 1941—43 festgestellt. Damals war der Fettgehalt der Muttermilch 25 % kleiner als in normalen Jahren, und der Kaloriengehalt war um 120 Kalorien kleiner als der von YLPPÖ im Jahre 1928 erhaltene. In Finnland war die Lebensmittellage Anfang des Jahres 1942 am schlechtesten. Mit der rationierten Nahrungsmenge war es damals schwer den Kalorienbedarf zu befriedigen, insbesondere in den Städten, in welchen von Zeit zu Zeit wegen Transportschwierigkeiten nicht einmal die rationierten Lebensmittelmengen zu bekommen waren (SIMOLA). Im Frühjahr 1941 stellte TURPEINEN in seinen an Volksschulkindern durchgeführten Untersuchungen Vitaminmangel fest, insbesondere A- und C-Hypovitaminose. Nicht alle Kinder litten in gleichem Masse unter der spärlichen Kalorienmenge, denn z.B. die im Spielalter stehenden Kinder bekamen in den Kindergärten eine zusätzliche Mahlzeit. Die Schulkinder hatten diesen Vorteil nicht, sondern für ihre Schulmahlzeiten wurde ein Teil der Kartenrationen benutzt. MALMIVAARA hat bei seiner Untersuchung über die Längen- und Gewichtszunahme der helsinkier Kinder festgestellt, dass sowohl bei den Mädchen als auch bei den Knaben der Gewichtswert während der Kriegsjahre zum grössten Teil kleiner war als der entsprechende Wert aus den Jahren 1938—39. Am meisten kamen Abweichungen im Gewicht während der Jahre 1942—1944 vor. Desgleichen hatte der Längenzuwachs, insbesondere bei den 8—13-Jährigen, nach dem Winterkrieg sturzartig abgenommen. In den letzten Jahren, auch schon unmittelbar nach dem Kriege, ist der Längenzuwachs wieder gestiegen. Aus den Folgen des ersten Weltkrieges hat HORNBORG im Jahre 1921 bei uns festgestellt, dass er sowohl auf das Längen- und Gewichtswachstum als auch auf die geistigen Leistungen der Volksschulkinder ungünstig eingewirkt hatte.

Nach der Pubertätszeit, eigentlich schon nach dem Auftreten der Menarche, wird die Nahrungssituation für die Mädchen leichter, denn beim menstruierenden Mädchen ist der Stoffwechsel

bedeutend niedriger als beim nicht menstruierenden (DROESE und ROMINGER).

Im Hinblick auf die mannigfaltigen Belastungen der Kriegszeit untersuchte ich, ob in Finnland im Menarchealter der Mädchen eine Veränderung stattgefunden hatte, und wenn dies der Fall war, in welcher Richtung dieselbe ging. Mein Material war gerade unter Berücksichtigung des Krieges so gewählt worden, dass es die im Jahre 1923 geborenen und jüngeren Altersklassen umfasste. Der Herbst 1939 war, obwohl er eine aussergewöhnliche Zeit darstellte, für die Mädchen jedoch nicht besonders anstrengend gewesen, sodass er wohl kaum Einfluss auf dass Menarchealter gehabt haben dürfte. Die Zeit des Zwischenfriedens habe ich als Kriegszeit gerechnet.

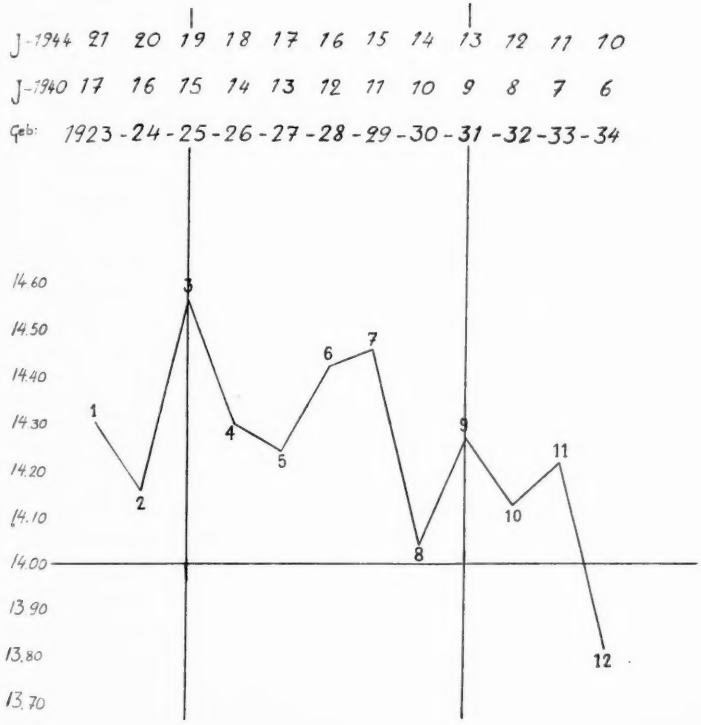
Mein Material teilte ich nach dem Geburtsjahr in Gruppen ein, diese wiederum in Untergruppen nach dem Menarchealter, und für diesen Untergruppen berechnete ich die Prozentzahlen. Tabelle Nr. 1. Beim Menarchealter habe ich eine Gruppierung befolgt, wonach z.B. als 14-jährig diejenigen Mädchen bezeichnet werden, bei welchen die Menarche zwischen 13,6 und 14,5 Jahren auftrat. Die Zahlen verteilen sich ziemlich gleichmässig innerhalb der normalen Grenzen. Ausserdem zeichnete ich eine Kurve nach dem Geburtsjahr, indem ich das Material dem Menarchealter gemäss einteilte. Figur Nr. 2.

Im Jahre 1940 waren die 1923 geborenen Mädchen 17-jährig und die 1933 geborenen 7-jährig. Nach Abschluss des Krieges im Jahre 1944 waren die 1923 geborenen 21 Jahre alt und die 1933 geborenen 11 Jahre. Man sollte annehmen, dass innerhalb dieser Grenzen alle diejenigen Platz finden, auf welche der Krieg Einfluss gehabt haben könnte. In meiner Tabelle Nr. 1 habe ich mit zwei Stufenlinien die Kriegsjahre hervorgehoben, und zwar links beim Jahre 1940 und rechts, weiter oben, beim Jahre 1944. Der so abgetrennte Teil ist im unteren Abschnitt vollständig, wo er die im Jahre 1934 Geborenen bis zum Schluss umfasst. Hier haben wir es allerdings mit zwei zu jungen Altersklassen zu tun, aber von diesen kommen auf das abgetrennte Gebiet nur diejenigen, bei welchen die Menarche im Alter von unter 12 Jahren aufgetreten ist, und deren Prozentzahlen so klein sind, dass sie beim Vergleich keine Bedeutung haben dürften. Weiter oben, bei den

Tabelle Nr. 1.

GEBOREN J. / MENARCHEALTER	J-1944														
	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37
19	1.6	–	1.4	0.6	0.8	0.8	–	0.4	–	–	–	–	–	–	–
18	5.0	3.3	6.2	1.8	0.9	–	2.0	0.2	0.2	0.1	–	–	–	–	–
J-1940															
0.5 17	5.0	6.0	4.1	7.6	5.6	5.6	6.4	4.1	4.3	2.2	0.6	–	–	–	–
0.6 16	11.0	9.3	13.1	8.2	9.4	14.1	14.7	11.3	13.4	10.4	8.7	2.1	–	–	–
15	14.9	17.3	24.1	21.8	21.4	24.6	24.7	19.8	24.7	27.4	27.3	24.1	8.5		
14	25.4	28.7	22.8	30.6	30.8	31.5	27.8	23.7	29.2	29.9	32.3	40.4	41.0	26.6	
13	26.0	22.0	19.3	18.8	22.6	16.1	15.0	29.8	19.1	20.4	22.7	22.6	36.7	51.4	43.8
12	8.3	10.0	6.9	9.4	6.8	5.7	7.4	9.3	8.0	8.1	7.1	9.5	11.9	19.9	49.3
0.5 0.6 11	1.7	2.7	2.1	1.2	1.7	1.6	2.0	1.4	1.1	1.2	0.9	1.1	1.5	2.1	6.9
10	1.1	0.7	–	–	–	–	–	–	–	0.3	0.4	0.2	0.4		
ANZAHL	187	150	245	170	234	248	299	486	467	689	759	819	793	287	73

Figur Nr. 2.



älteren Altersklassen, ist das abgestufte Gebiet unvollständig. Zwei ältere Altersklassen, die in den Jahren 1922 und 1921 Geborenen, hätten die Tabelle vervollständigt, da deren Anteil aber nur diejenigen betroffen hätte, welche im Alter von 21 und 22 Jahren ihre erste Menstruation gehabt hatten, und da die Tabelle auch so schon klare Beweise gibt, habe ich es für unnötig erachtet, diese Vervollständigung vorzunehmen. Aus der Tabelle geht hervor, dass während der Kriegszeit oder unmittelbar danach keine beträchtlichere Verschiebung des Menarchealters nach unten oder oben stattgefunden hat. Wenn man die Prozentzahlen innerhalb der Stufenlinien prüft und sie mit denjenigen ausserhalb vergleicht, so stellt man fest, dass sie ungefähr gleich sind. Tabelle Nr. 1.

Die im Jahre 1923 geborenen Mädchen waren beim Ausbruch des Krieges 17 Jahre alt, so dass derselbe keinen Einfluss mehr auf sie haben konnte, das gleiche betrifft die 1924 geborenen, da dieselben im Jahre 1940 16-jährig waren. Die Mädchen, deren Geburtsjahr 1925 ist, waren bei Kriegsausbruch 14,6—15,5 Jahre alt. In dieser Altersgruppe ist die Anzahl derjenigen, welche 14-jährig zum erstenmal menstruierten, im Vergleich zu den vorhergehenden Jahrgängen kleiner: $25{,}4 \pm 3{,}2$ — $28{,}7 \pm 3{,}7$ — $22{,}8 \pm 3{,}5$ %, und wiederum sofort nach Beginn des Kriegszustandes, als sie 15-jährig waren, entsprechend grösser: $14{,}9 \pm 2{,}6$ — $17{,}3 \pm 3{,}0$ — $24{,}1 \pm 3{,}6$ %. Die Differenz zwischen $24{,}1 \pm 3{,}6$ und $17{,}3 \pm 3{,}0$ ist nur $6{,}8 \pm 4{,}7$, also nicht wirklich. Ausserdem sind von dieser Jahresklasse 6,2 % beim Auftreten der Menarche 18 Jahre alt gewesen. In der Figur Nr. 2 sieht man bei ihnen einen ziemlich steilen Anstieg, und die Differenz zum Menarchealter der vorhergehenden Gruppe, der 1924 geborenen, ist signifikant: $0{,}42 \pm 0{,}19$ Jahre. In meinem ganzen Material ist auch bei der Jahresklasse 1925 dieses Alter am höchsten: $14{,}57 \pm 0{,}14$ Jahre. Unter den im Jahre 1926 Geborenen war die Menarche im Alter von 14 Jahren bei $30{,}6 \pm 3{,}5$ % aufgetreten. Obwohl diese Zahl im Vergleich zur Zahl der gleichaltrigen aus dem vorhergehenden Jahrgang ziemlich viel grösser ist, so besteht in Wirklichkeit keine signifikante Differenz: $7{,}8 \pm 4{,}9$ %. In der Kurve ist bei diesen Mädchen das Menarchealter im Vergleich zur vorhergehenden Jahresklasse gesunken, aber die Differenz ist auch hier nicht signifikant: $0{,}27 \pm 0{,}18$ Jahre. Die im Jahre 1927 Geborenen waren bei Kriegsausbruch 13-jährig. In Bezug auf sie weist die Prozenttabelle keine besonders abweichenden Zahlen auf, und auch in der Kurve ist nur ein geringes Sinken wahrzunehmen. Bei diesen Mädchen ist die Differenz im Menarchealter im Vergleich zu den anderen entsprechenden Zahlen nicht signifikant. Die im Jahre 1928 geborenen Mädchen waren bei Beginn des Krieges 12-jährig. Im Alter von 13 Jahren trat unter ihnen bei einer verhältnismässig kleinen Prozentzahl die Menarche auf, nur $16{,}1 \pm 2{,}3$ %, aber trotzdem ist der Unterschied im Vergleich zum vorhergehenden Jahrgang nicht signifikant, er ist nur $6{,}5 \pm 3{,}6$ %. In der Kurve haben die 1928 geborenen einen kleinen Anstieg, aber die Differenzen im Vergleich zur vorhergehenden

und zur folgenden Gruppe sind nicht wirklich. Die Prozentzahlen der im Jahre 1929 geborenen sind gleichmässig, Abweichungen vom allgemeinen Niveau kommen nicht vor. Sehr jung oder älter als gewöhnlich haben von ihnen nicht mehr als der gewöhnliche Prozentsatz menstruiert. Die Kurve zeigt an ihrer Stelle ständigen Anstieg, aber zu keiner früheren Gruppe ist der Unterschied signifikant. Am grössten von den bis jetzt durchgegangenen Jahrgängen ist die 486 Mädchen umfassende Gruppe der im Jahre 1930 geborenen. Hier fällt die Prozentzahl der im Alter von 13 Jahren gereiften Mädchen auf: $29{,}8 \pm 2{,}0$ %. Der Unterschied zwischen dieser und der entsprechenden Zahl der 1929 geborenen ist gross: $14{,}8 \pm 2{,}9$ %. Warum in dieser Jahresklasse so viele Mädchen 13-jährig menstruierten, ist schwer zu sagen. Der Krieg hatte schon 3 Jahre gedauert und stand damals in einem ziemlich ruhigen Stadium, so dass äusserliche Ursachen nicht zu finden sind. Die Zahl wurde auch so untersucht, dass andere das Menarchealter vermindernde Momente in Betracht gezogen wurden, wie die schwedische Sprache, das Leben in der Stadt und die geistige Arbeit des Vormundes, aber keine von diesen unteren Gruppen war hier besonders reichlich vertreten, so dass also ein anderer Faktor, den ich nicht zu erklären weiss, Einfluss gehabt haben dürfte. In der Kurve ist an der Stelle dieses Jahrgangs ein beträchtliches Sinken wahrzunehmen. Das Menarchealter ist $14{,}04 \pm 0{,}02$ Jahre. Die Differenz im Vergleich zum Jahrgang 1925 ist $0{,}53 \pm 0{,}16$ Jahre, zum Jahrgang 1928 $0{,}38 \pm 0{,}11$ und zu den im Jahre 1929 geborenen $0{,}14 \pm 0{,}11$ Jahre. Auch im Vergleich zum folgenden Jahrgang ist die Differenz signifikant: $0{,}23 \pm 0{,}01$ Jahre. Der Jahrgang 1931 ist schon sehr jung, bei Kriegsausbruch 9-jährig. Unter ihnen lässt sich auch kein Einfluss des Krieges beobachten, sofern man nicht in Betracht zieht, dass sofort im Jahre 1944, also bei Kriegsende, ihre Prozentzahl ziemlich gross ist, aber zu diesem Zeitpunkt waren sie ja gerade 14-jährig, in welchem Alter die Menarche oft eintritt. Der Frieden im Jahre 1944 kam auch nicht plötzlich, und wirkte nicht sofort entscheidend auf unsere Situation ein, so dass kaum anzunehmen ist, dass er das Menarchealter beschleunigend oder verzögernd beeinflusst hätte. Die folgenden Altersklassen sind so jung, dass die Einwirkung des Krieges in Bezug auf sie das Menarchealter

nicht verändert zu haben scheint. Aus der Kurve geht hervor, dass schon die im Jahre 1934 geborenen eine zu junge Altersklasse sind, denn ihr Menarchealter ist beträchtlich unter 14 Jahre gesunken.

Wenn man die auf beiden Seiten der Stufenlinien befindlichen Zahlen miteinander vergleicht, so hat es nicht den Anschein, als ob der Kriegsausbruch, der Kriegszustand oder der Friedensschluss das Menarchealter unserer Mädchen wesentlich beeinflusst hätte.

Besonders die Deutschen haben die Folgen des Krieges für die im Entwicklungsalter Stehenden untersucht. Die Verminderung der Widerstandskraft und die Verschlechterung der hygienischen Verhältnisse sowie ferner der Hunger sind ihres Erachtens die Ursache für die Verzögerung der Entwicklung bei den im Pubertätsalter befindlichen jungen Menschen (SCHLESINGER, HÄBERLIN). Die Folgen des ersten Weltkrieges erstreckten sich so weit, dass erst die in den Jahren 1921—1922 geborenen Mädchen im Wachstum auf dem vorkriegszeitlichen Niveau standen (WOLF). GRIMM stellte fest, dass die im Jahre 1932 geborenen Mädchen am schwersten unter dem zweiten Weltkrieg gelitten hatten, denn sie hatten einen Defizit von 19,2 % im Menarchealter im Vergleich zu den Friedensjahren. Im Jahre 1949 behauptete NEVINNY-STICKEL, dass der Krieg den Verjüngungsprozess der Menarche nicht unterbrochen habe, obwohl diejenigen Jahrgänge, welche gegen Ende der Weltkriege im Pubertätsalter standen, in diesem Stadium beträchtlich verspätet waren. Insbesondere bei denjenigen Mädchen, welche im Jahre 1945, als der Krieg am schwersten war, im Pubertätsalter standen, verspätete sich die Menarche um ein Jahr.

In Deutschland war die durch den Krieg verursachte Situation eine ganz andere als bei uns. In Finnland war die Lebensmittellage zwar schwer, aber direkte Hungersnot bestand nicht. Desgleichen, obwohl die psychischen Belastungen gross waren, wirkten die Natur und die Zähigkeit unseres Volkes sicherlich ausgleichend, so dass äussere Zeichen, wie Erhöhung des Menarchealters infolge der Kriege nicht wahrzunehmen waren.

Auf Grund meiner Untersuchung dürfte es als offenbar angesehen werden können, dass der Einfluss der Kriegsjahre auf die

Jugend des finnischen Volkes, insbesondere auf die im Entwicklungsalter stehenden jungen Mädchen, nicht so verderblich gewesen ist, wie er hätte sein können, sondern sie haben ihn so gut überstanden, dass das Menarchealter gleichgeblieben, ja sogar ein wenig gesunken ist.

3. Der Einfluss der Sprachen- und Rassenverteilung auf das Menarchealter sowie das Menarchealter bei der verschiedenen finnischen Stämmen.

Rassisch gehört das finnische Volk in der Hauptsache zur ostbaltischen Völkergruppe, mit welcher sowohl nordische Rasse als auch Lappen vermischt sind. In reiner Form trifft man Vertreter der beiden Hauptgruppen, sowohl der ostbaltischen als auch der nordischen Rasse, nur noch selten. Die ersteren in Häme (ca. 14 %), die letzteren in den Schären und in der Küstengegend von Pohjanmaa (PESONEN). Die ostbaltische Rasse soll ca. im Jahre 100 n. Chr. in das Land eingedrungen sein. Ahvenanmaa ist ein Kapitel für sich, es ist nicht von der finnischen Rasse besiedelt gewesen, sondern seine Bewohner sind Germanen. Von Schweden ist Bevölkerung auf die Finnische Halbinsel während der Kreuzzüge und danach, d.h. im 11. Jahrhundert übergesiedelt. Die Auffassung, dass die Karelier und Savolaxer ursprünglich zu einer anderer Rasse gehörten als die übrigen Finnen, hat man heutzutage verworfen (SALMO, ROSCHIER).

Die schwedische Sprache ist lange Zeit Amtssprache gewesen, und die Gebildeten gebrauchten sie noch bis zum Anfang unseres Jahrhunderts, weshalb die sprachliche Verteilung nicht der rassischen Verteilung entspricht. Auf jeden Fall lassen sich in Finnland grosse Konstitutionsunterschiede zwischen den in den verschiedenen Gegenden wohnenden Menschen beobachten (PESONEN). Die Bevölkerung der westlichen Teile ähnelt mehr den Germanen als die weiter nördlich und östlich wohnende (WESTERLUND), aber KAJAVA vermutete, dass auch die sozial-hygienischen Verhältnisse bei diesem Unterschied grosse Bedeutung hätten, da in Westfinnland die Verhältnisse früher geordnet waren als anderwärts in unserem Lande. Eine eigentliche Rasseneinteilung gibt es in Finnland nicht.

Nach der im Jahre 1950 durchgeführten Volkszählung gab es in Finnland finnischsprechende Finnen 91,2 % von der ganzen Bevölkerungszahl und schwedischsprechende 8,6 %, zu anderen Sprachengruppen gehörten 0,2 %. Diese letzteren habe ich bei der Sammlung meines Materials zu vermeiden gesucht.

Mein Material enthält Vertreter aus beiden Sprachengruppen. Finnischsprechende finnische Mädchen gehören 2823 oder 92,4 % dazu und schwedischsprechende 231 oder 7,6 %.

Die Bedeutung der Rasse für das Menarchealter wird allgemein als offenbar angesehen. Von den Juden ist oftmals statistisch nachgewiesen worden, dass sie im grossen und ganzen in gleicher Weise menstruieren, unabhängig davon, wo sie wohnen (Raciborski). Desgleichen ist festgestellt worden, dass die von englischen Eltern abstammenden in Indien geborenen Mädchen ihre Menarche auf englische Weise bekommen, d.h. früher als die Mädchen in England, aber später als die Indierinnen (Roberton). Yamasaki hat in Japan verschiedene Werte für das Menarchealter bei den Frauen der verschiedenen Rassen seines Landes berechnet. Nach Bolk tritt in Holland bei den Frauen der blonden nordischen Rasse die Menarche 10 Monate früher ein als bei denjenigen der alpinen Rasse. Die alpine und die nordische Rasse verglich auch Stein in Deutschland miteinander, er konnte aber keinen Unterschied im Menarchealter dieser beiden Rassen feststellen. Amerika bietet ja den denkbar günstigsten Boden für die Untersuchung des Anteils der Rasse am Menarchealter. Das Material von Araujo umfasst sowohl Weisse als auch Farbige, aber obwohl er gesondert das Menarchealter für die Weissen, Mulatten und Neger berechnete, so konnte er keinen Unterschied in dieser Beziehung zwischen diesen drei Gruppen feststellen. Araujo vermutet, dass die seinerzeit aus Portugal nach Brasilien übergesiedelten Weissen nunmehr in ihrem Organismus den gleichen Faktor besitzen, den die Neger haben, und dass sie deshalb auf andere Weise reagieren als die Weisshäutigen in Europa. Kennedy nahm demgemäss ach an, dass die sozialen Verhältnisse, die Nahrung, welche vom Klima abhängig ist, und die Umgebung die Faktoren seien, welche auslösend auf den Reifungsprozess wirken.

Die Abhängigkeit der Menarche von den rassischen Eigenschaften ist auch mit Hilfe der Erblichkeit bewiesen worden. In seinen Zwillingsforschungen hat Petri sowohl ein- als auch zweieiige Zwillinge miteinander verglichen, wobei die äusseren Verhältnisse gleich waren. Bei den eineiigen Zwillingen war der Unterschied im Auftreten der ersten Menstruation 2,8 Monate,

bei den zweieiigen 12 Monaten, und bei Geschwistern 14,4 Monate. Desgleichen ist festgestellt worden, dass das Menarchealter der Töchter abhängig ist vom entsprechenden Alter der Mütter (BREIPOHL, STUART), und dass zwischen dem Menarchealter der Geschwister ein deutliches Verhältnis besteht (GOULD und GOULD). Es ist auch behauptet worden, dass der Einfluss der Umgebung nicht besonders gross sei, sondern dass gerade die hereditären Eigenschaften bei dieser Erscheinung am wichtigsten seien. LINTZ und MARKOW behaupten, dass die Geschwister ihre erste Menstruation im verhältnismässig gleichen Alter nicht deshalb bekommen, weil die Verhältnisse die gleichen sind, sondern weil die vererbten Eigenschaften so ähnlich sind.

In Finnland hat HEINRICIUS schon im Jahre 1882 gesondert das Menarchealter der finnisch- und schwedischsprechenden Frauen in unserem Lande berechnet, stellte darin aber keinen Unterschied fest. *Malmio* hingegen erhielt folgende Werte:

	Menarchealter
Finnischsprechende Arbeiterfrauen	16,05 ± 0,10 Jahre
Schwedischsprechende Arbeiterfrauen	15,56 ± 0,15 „

Im Menarchealter der finnisch- oder schwedischsprechenden Frauen unseres Landes besteht hier ein beträchtlicher Unterschied: 0,49 ± 0,18 Jahre bei den letzteren früher als bei den ersteren. Es sei erwähnt, dass ungefähr zur gleichen Zeit, im Jahre 1906, in Schweden das Menarchealter 15,72 ± 0,03 Jahre war (LENNÉR).

Bei meinen Untersuchungen erhielt ich die unten aufgeführten Werte für das Menarchealter bei den beiden Sprachengruppen, ohne die Gesellschaftsklasse oder die Vermögensverhältnisse in Betracht zu ziehen:

	Anzahl	Menarchealter
Finnischsprechende	2823	14,28 ± 0,08 Jahre
Schwedischsprechende	231	13,85 ± 0,09 „

Nach dieser im Jahre 1950 ausgeführten Berechnung bekamen die schwedischsprechenden Mädchen in unserem Lande ihre erste Menstruation 0,43 ± 0,13 Jahre früher als die finnischsprechen-

den. In MALMIOS Material sind die Werte aus Menarchefällen vom Ende des vorigen Jahrhunderts berechnet, meine eigenen Werte wiederum entsprechend aus Menarchefällen zwischen den Jahren 1933—1942. In MALMIOS Material war der Unterschied 5,9 ± 2,2 Monate, in meinem 5,2 ±1,6 Monate, so dass sich also keine wesentliche Verschmälerung im Verhältnis des Menarchealters bei den finnisch- und schwedischsprechenden Mädchen während diesem Zeitabschnitt feststellen lässt.

Nach LENNÉR war in Schweden das Menarchealter im Jahre 1940 14,49 ± 0,03 Jahre, bei uns in Finnland reiften die schwedischsprechenden Mädchen nach den im Jahre 1950 auf Grund meines Materials gemachten Berechnungen 0,64 ± 0,10 Jahre früher als die Mädchen in Schweden.

Bedauerlicherweise habe ich in der Literatur keine Angaben über das Menarchealter bei den uns verwandten Esten aus den letzten Jahrzehnten gefunden, ebensowenig habe ich entsprechende Mitteilungen über die übrigen Völker finnischen Stammes gesehen. Die letzten Werte, welche ich aus der russischen Literatur hier gefunden habe, stammten aus dem Jahre 1894. Bei den zu den verschiedenen Sprachgruppen gehörende Knaben ist auch ein Unterschied im Auftreten der ersten Ejakulation wahrzunehmen. Nach den Untersuchungen von WESTLING geschieht dies bei den schwedischsprechenden finnischen Knaben durchschnittlich früher als bei den finnischsprechenden.

Das Menarchealter war bei den finnischsprechenden Mädchen in Finnland 14,28 ± 0,08 Jahre und bei den schwedischsprechenden 13,85 ± 0,09 Jahre, Differenz 0,43 ± 0,13 Jahre.

Da zwischen dem Menarchealter der finnisch- und der schwedischsprechenden Frauen in unserem Lande ein beträchtlicher Unterschied bestand, nämlich in meinem Material bei den ersteren 14,28 ± 0,08 Jahre und bei den letzteren 13,85 ± 0,09 Jahre, habe ich mein Material auch in Bezug auf entsprechende Unterschiede zwischen den verschiedenen Stämmen untersucht.

Stämmenweise hat auch OINONEN im Jahre 1952 ihr Material von finnischen Frauen untersucht und stellte dabei Unterschiede in der Körpergrösse und den Bäckenmassen der Frauen aus den verschiedenen Stämmen fest. Bei den Frauen aus Savo und Kar-

jala war die Körperlänge über 2 cm kleiner als bei den Frauen der westlichen Stämmen.

Aus manchen Landesteilen habe ich nur ein beschränktes Material. Eine Karte, aus welcher die von mier befolgte Einteilung nach den Stämmen hervorgeht, ist dieser Publikation beigefügt. Die Abgetretenen Gebiete liegen zwischen den beiden Grenzlinien. Die Verteilung auf die verschiedenen Stämme, getrennt in Stadt- und Landbevölkerung, ist folgende:

A. Stadtbevölkerung:

	Anzahl	Menarchealter
1. Uusimaa (Nyland)	491	13,94 ± 0,06 Jahre
2. Varsinais-Suomi (Eg. Finland), Satakunta (Satakunda) & Ahvenanmaa (Åland)	259	13,83 ± 0,08 „
3. Häme (Tavastland)	145	14,12 ± 0,11 „
4. Savo (Savolax)	108	14,60 ± 0,20 „
5. Karjala (Karelen)	318	14,08 ± 0,08 „
6. Pohjanmaa (Österbotten) .	162	14,25 ± 0,11 „

B. Landbevölkerung:

	Anzahl	Menarchealter
1. Uusimaa	94	14,37 ± 0,14 Jahre
2. Varsinais-Suomi, Satakunta & Ahvenanmaa	190	14,33 ± 0,09 „
3. Häme	145	14,22 ± 0,12 „
4. Savo	508	14,66 ± 0,06 „
5. Karjala	247	14,43 ± 0,09 „
6. Pohjanmaa	275	14,27 ± 0,08 „

Ein Unterschied zwischen dem Menarchealter unter der Stadt- und der Landbevölkerung kommt nur in folgenden Gruppen vor:

A. 1—B. 1	0,43 ± 0,16 Jahre
A. 2—B. 2	0,50 ± 0,13 „
A. 3—B. 3	0,35 ± 0,12 „ .

Unter der Stadtbevölkerung besteht Unterschied zwischen den Gruppen:

1—4	0,66 ± 0,21	Jahre
1—6	0,31 ± 0,13	„
2—3	0,29 ± 0,14	„
2—4	0,77 ± 0,22	„
2—5	0,25 ± 0,11	„
2—6	0,42 ± 0,14	„
3—4	0,48 ± 0,23	„
4—5	0,52 ± 0,22	„ ,

sowie bei der Landbevölkerung entsprechend:

2—4	0,33 ± 0,11	Jahre
3—4	0,44 ± 0,13	„
4—5	0,23 ± 0,11	„
4—6	0,39 ± 0,10	„ .

Wie man sieht, besteht zwischen der Gruppe Varsinais-Suomi, Satakunta und Ahvenanmaa ein deutlicher Unterschied im Menarchealter im Vergleich zu den übrigen mit Ausnahme der Stadtbevölkerung von Uusimaa. Hier dürfte das niedrigere Menarchealter der schwedischsprechenden Mädchen grossen Anteil haben, denn gerade in den westlichen Landesteilen ist die schwedische Sprache am reichlichsten vertreten. Die Landbevölkerung hingegen entwickelt sich nach meinem Material im allgemeinen nicht schneller, nur in Bezug auf Savo ist die Differenz signifikant.

Bei den Mädchen aus Savo ist in beiden Gruppen das Menarchealter am höchsten: in der Stadt 14,60 ± 0,20 Jahre und auf dem Lande 14,66 ± 0,06 Jahre.

Im allgemeinen ist angenommen worden, dass die Karelier bezüglich ihrer Entwicklung rückständiger seien als die Bevölkerung von Mittel- und Westfinnland. Wilskman stellte im Jahre 1920 bei seiner Untersuchung über Maximum- und Minimumzuwachs der Körperlänge fest, dass der grösste Teil der Minimawerte in Ostfinnland erhalten wurde, insbesondere aus der Stadt Viipuri, während wiederum die maximalen Zahlen in den westlichen Landesteilen angetroffen wurden. Nach Wilskmans Auffassung war dies sehr charakteristisch für die Verhältnisse in Finnland. Nach seiner Untersuchung fiel in Uusimaa das Maxi-

mum des Längenwachstums der Mädchen auf das 13.—14. Lebensjahr, in Karjala wiederum ca. ½—1 Jahr später, auf das 14.—15. Lebensjahr. Das Gleiche traf für die Gewichtszunahme zu, in Uusimaa fiel das Maximum auf das 13.—14. Lebensjahr, in Karjala erst im Alter von 14—15 Jahren. Da wir wissen, dass der Eintritt der ersten Menstruation gerade vom Maximum der Längen- und Gewichtszunahme abhängig ist, wären die karelischen Mädchen später gereift als die westfinnischen, ja sogar noch ein wenig später als die Mädchen aus Savo.

Das Menarchealter der Karelier war nach meinem Material bei der Stadtbevölkerung $14{,}08 \pm 0{,}08$ Jahre. Aus meinem gesamten Material berechnet war die entsprechende Zahl der Stadtbevölkerung $14{,}08 \pm 0{,}03$ Jahre. Nach meinem Material trat die erste Menstruation bei der karelischen Stadtbevölkerung im gleichen Alter ein wie durchschnittlich in ganz Finnland. Ebenso war das Verhältnis bezüglich der Landbevölkerung: Bei den Kareliern $14{,}43 \pm 0{,}09$ Jahre, während die entsprechende das ganze Land betreffende Zahl $14{,}46 \pm 0{,}04$ Jahre war. Im Vergleich zu den Mädchen aus Savo war der Unterschied zwischen der Stadtbevölkerung $0{,}52 \pm 0{,}22$ Jahre und bei der Landbevölkerung $0{,}23 \pm 0{,}11$ Jahre. Wenn wir es als wahrscheinlich ansehen, dass die Karelier rassisch den Savolaxern am nächsten stehen, und dass sie im Jahre 1920 gemäss den Längen- und Gewichtsmessungen von Wilskman hinter diesen zurückstanden, wennschon sie im Vergleich zu den anderen Stämmen den Savolaxern am nächsten kamen, so hätte man erwartet, dass bei den Kareliern die Menarche später eingetreten wäre als bei den Mädchen aus Savo, oder höchstens im gleichen Alter. Der Umstand, dass bei den karelischen Mädchen die erste Menstruation früher eintritt, kann meines Erachtens entweder bedeuten, dass die Karelier, welche lebhaft und zu Neuerungen geneigt sind (Nordenstreng) sich während der Selbständigkeit unseres Landes früher und schneller als der benachbarte Stamm entwickelt haben, oder dass der Krieg und die zwangsweisen Umsiedelungen mit ihren vielen Schwierigkeiten befördernd auf die Entwicklung der karelischen Mädchen eingewirkt haben.

Unter den finnischen Stämmen bestand kein grosser Unterschied im Menarchealter in meinem Material.

Bei den Mädchen aus Savo trat auf Grund meines Materials die erste Menstruation später ein als bei irgend einem anderen Stamm.

Die Kriegszeit und ihre Folgen haben die Entwicklung der Karelier nicht verlangsamt, eher umgekehrt.

4. Der Einfluss des Berufs auf das Menarchealter.

Die Tätigkeit der endokrinen Drüsen lässt sich durch allerlei Umstände beeinflussen, und daher können die Vermögensverhältnisse und die Bildungsstufe auf das Menarchealter Einwirkung haben. Nach dem Vermögen lässt sich heutzutage wenigstens in Finnland das Material sehr schwer einteilen, und meines Erachtens entspricht dies auch nicht dem Zweck. Die meisten unserer Autoren, ENGSTRÖM und MALMIO, haben ihre Einteilung darauf gegründet, ob die Patientin in einem allgemeinen oder privaten Krankenhaus oder in der privaten Sprechstunde gewesen war; aber heute kann man daraus nicht den gewünschten Schluss ziehen. Wenn man das Jahreseinkommen als Masstab benutzen würde, käme man vermutlich zum gleichen Resultat wie ENGELMANN um die Jahrhundertwende in Amerika, nämlich dass im Entwicklungsalter kein Unterschied besteht.

Schon vor Beginn unserer Zeitrechnung bekamen die gebildeten, vornehmen indischen und ägyptischen Frauen ihre erste Menstruation früher als die gewöhnlichen Frauen aus dem Volke (MILLS). Am Ende des vorigen Jahrhunderts wurde von Neuem festgestellt, dass bei den sozial besser gestellten Mädchen im Vergleich zu den anderen Frauen die Menarche früher eintrat.

Im allgemeinen ist man sich über die Faktoren, welche bei den schlechter situierten Frauen die spätere Reife bedingen, einig gewesen: Die schlechteren Lebensbedingungen, ungenügende und einseitige Nahrung und der Zwang, schon früh schwere Arbeit zu verrichten. Die frühe Entwicklung und die kürzere Pubertätszeit der höheren Kreise ist den besseren äusseren Verhältnissen, den geistigen Bestrebungen sowie dem unruhigen und hitzigen Leben zugeschrieben worden (RACIBORSKI, ENGELMANN, ARON). MALMIO war der Ansicht, dass das Studium kein frühes Reifen verursachte.

sondern diese Mädchen reiften deshalb früh, weil sie zu geistigen Leistungen imstande waren, d.h. die Ursache war bei beiden die gleiche. ENGSTRÖM stellte schon im Jahre 1894 fest, dass auch in Finnland die Mädchen aus den höheren Kreisen früher menstruierten als die der Arbeiterklasse. Bei MALMIO entschieden die Vermögensverhältnisse die soziale Stellung. Andererseits waren auch die Verhältnisse am Ende des vorigen Jahrhunderts, aus welcher Zeit MALMIOS Material stammt, so, dass der geistige Arbeiter besser besoldet war als derjenige, welcher körperliche Arbeit verrichtete. MALMIO beobachtete schon den Aufstieg der Arbeiterklasse und die teilweise darauf beruhende allgemeine Verminderung des Menarchealters. Um sein Material vielseitiger zu gestalten, nahm MALMIO den grössten Teil, die Angaben über Arbeiterfrauen, aus den Krankenberichten der Entbindungsanstalt und des gynäkologischen Krankenhauses, die Angaben über die "Vornehmen" aus einer helsinkier Privatklinik, wo er nur die auf den teuersten Plätzen gewesenen auswählte, sowie als Mittelstufe die Diakonissenanstalt. MALMIO berechnete folgende Werte:

	Menarchealter	
Arbeiterfrauen	16,09 ± 0,06	Jahre
Mittelstand	15,92 ± 0,14	„
Höhere Kreise	15,02 ± 0,05	„

Der Unterschied zwischen dem Menarchealter der Arbeiterfrauen und den Frauen des Mittelstandes ist nicht signifikant: 0.17 ± 0,15 Jahre. Der Unterschied zwischen den Arbeiterfrauen und den Frauen der höheren Kreise ist 1,07 ± 0,07 Jahre, sowie zwischen dem Mittelstand und den höheren Kreisen 0,90 ± 0,15 Jahre. MALMIO legte sehr grosses Gewicht auf die Heredität. Er vermutet, dass diejenigen Frauen — in seinen Fällen also die sozial besser gestellten —, welche früher reifen, auch früher gebären und diese Fähigkeit auf ihre Kinder vererben, auf welche Weise die Anzahl der früher Reifenden ständig vermehrt wird. Andererseits wiederum nimmt MALMIO an, dass eine Frau, welche schnell die Geschlechtsreife erlangt, auch schnell die geistige Reife erreicht und daher imstande ist, sich früher und besser eine ökonomische Stellung zu verschaffen. Der von VUORI berechnete

bedeutend niedrigere Wert des Menarchealters bei Schülerinnen der Mittelschulen im Vergleich zu den Seminaristen brachte Salo zu der Vermutung, dass die Ursache für diesen Unterschied im Einfluss der Schulerziehung zu suchen sei. Es ist aber wahrscheinlicher, dass der Unterschied durch die Methode bei der Sammlung des Materials bedingt ist, denn die Mittelschülerinnen gehörten einer jüngeren Altersklasse an, und ausserdem waren diejenigen, bei welchen die erste Menstruation nicht aufgetreten war, aus den Berechnungen weggeblieben.

Bei meiner Arbeit habe ich in erster Linie den Beruf des Vormundes berücksichtigt, weil ich der Ansicht war, dass dieser besser die soziale Stellung des betreffenden Mädchens anzeigte als ein möglicherweise schon erworbener eigener Beruf. Nach diesem Prinzip teilte ich mein Material in zwei Hauptgruppen ein, nämlich die geistigen und die körperlichen Arbeiter. Das im Hinblick auf die soziale Stellung eingeteilte Material umfasst 2786 Fälle. Versuchsweise teilte ich die beiden obengenannten Gruppen noch in zwei Unterabteilungen ein, nämlich die stelbständig Arbeitenden und diejenigen, welche in untergeordneter Stellung arbeiten. Es erwies sich aber, dass der Unterschied dieser beiden so erhaltenen Unterabteilungen im Vergleich zur Hauptgruppe nicht signifikant ist. Die nach dem Beruf berechneten Werte des Menarchealters waren folgende:

	Anzahl	Menarchealter
Geistige Arbeit Verrichtende	1192	$14{,}03 \pm 0{,}04$ Jahre
Körperliche Arbeit Verrichtende ..	1594	$14{,}39 \pm 0{,}04$ „

Die Differenz ist tatsächlich: $0{,}36 \pm 0{,}05$ Jahre.

Aus dem Obigen geht hervor, dass die geistige Arbeit verrichtenden Frauen oder die Töchter von geistige Arbeit verrichtenden Personen ihre erste Menstruation früher bekommen als diejenigen, welche körperliche Arbeit verrichten. Es hat also den Anschein, dass die Art der Arbeit oder die soziale Stellung trotz unseres demokratischen Gemeinwesens immer noch auch dem Menarchealter ihren Stempel aufdrückt. Möglicherweise hat auf den niedrigeren Mittelwert des Menarchealters unter den geistige

Arbeit Verrichtenden die entsprechende niedrigere Zahl der Städter Einfluss, vielleicht ist auch der Anteil der schwedischsprechenden Bevölkerung daran beteiligt, aber in unserem Lande liegt keine allgemeinstatistisch berechnete Prozentzahl der Schwedischsprechenden im Beamtenstand vor, so dass sich auf Grund der von mir erhaltenen Zahlen keine Kontrolle vornehmen lässt.

Die von MALMIO berechneten Werte stammen von am Ende des 19. Jahrhunderts geborenen Frauen, meine wiederum von in den Jahren 1923—33 geborenen. Wenn wir die Resultate miteinander vergleichen, stellen wir folgendes fest:

MALMIO

	Menarchealter
Arbeiterfrauen	16,09 ± 0,05 Jahre
Obere Kreise	15,02 ± 0,05 „

SIMELL

	Menarchealter
Körperliche Arbeit	14,39 ± 0,04 Jahre
Geistige Arbeit	14,03 ± 0,04 „

Aus dem Obigen sieht man deutlich, wie die Entwicklung in unserem Jahrhundert fortschrittlich auf unser Volk eingewirkt hat. MALMIO hat allerdings sein Material im Hinblick auf die Vermögensverhältnisse eingeteilt, ich wiederum nach der Art der Arbeit, und heutzutage kann man nicht von der Annahme ausgehen, dass der geistige Arbeiter ökonomisch besser gestellt sei als der körperliche. Gewisse Hinweise gibt der Vergleich aber doch. Der Unterschied zwischen den beiden von MALMIO berechneten Werten ist 1,07 ± 0,07 Jahre, zwischen meinen Werten wiederum 0,36 ± 0,05 Jahre. Die Differenz, wenn man sie als solche nehmen will, ist also um 0,71 ± 0,08 Jahre oder 8,52 ± 0,96 Monate kleiner geworden.

Wenn nach MALMIOS Vermutung das niedrigere Menarchealter auf hereditärem Wege das Reifealter ständig herabgesetzt hätte, und wenn die so erreichte frühe Reife grössere geistige und ökonomische Errungenschaften bewirkt hätte, so wäre zu erwarten

gewesen, dass die Differenz, wennschon die sozialen Verhältnisse sich verändert haben, doch gleich geblieben oder nur wenig schmäler geworden wäre. Dies ist jedoch nicht der Fall. Meinerseits möchte ich annehmen, dass die äusseren Faktoren, das Miljeu, bedeutenden Einfluss haben. Alles, was die Entwicklung in unserem Jahrhundert mit sich gebracht hat, die Hygiene, die soziale Fürsorge, der Schulzwang, die Arbeiterbewegung usw., haben zu einem Ausgleich des Menarchealters zwischen den verschiedenen sozialen Klassen geführt.

In Schweden hat LENNÉR im Jahre 1944 das Menarchealter im Hinblick auf die Vermögensverhältnisse untersucht und stellte dabei fest, dass die sozial besser gestellten Mädchen nahezu ein halbes Jahr früher menstruieren als die anderen Mädchen. Bei uns ist der Unterschied nur 4,3 ± 0,6 Monate.

Aus meinen Untersuchungen hat sich ergeben, dass *in Finnland die geistige Arbeit Verrichtenden oder ihre Töchter die erste Menstruation früher bekommen als die körperlich Arbeitenden oder ihre Töchter.* Der Unterschied ist 0,36 ± 0,05 Jahre. Die Differenz ist früher bedeutend grösser gewesen und ihre Verschmälerung dürfte auf die Entwicklung der sozialen Verhältnisse sowie den für unser Jahrhundert charakteristischen hitzigen Lebenstakt zurückzuführen sein. Ein wichtiger Faktor dürfte es auch sein, dass die Kluft zwischen den verschiedenen Gesellschaftsklassen im Verschwinden begriffen ist. Gleichzeitig ist auch festgestellt worden, dass während einer Zeit von über 25 Jahren der Unterschied im Menarchealter zwischen den sogenanten gebildeten und ungebildeten Klassen beträchtlich kleiner geworden ist.

5. Der Einfluss des Wohnorts und der Umsiedelungen auf das Menarchealter.

Der Einfluss des Wohnorts auf das Menarchealter ist bekannt. Die alten Juden beobachteten schon zur Zeit des Talmuds den Unterschied im Menarchealter der Stadt- und der Landmädchen. Ihres Erachtens war das Baden ein ausschlaggebendes Moment, sie nahmen nämlich an, dass die bei den Stadtmädchen üblichen heissen Bäder die Reifung förderten (MILLS). Später ist immer

von Neuem festgestellt worden, dass die Stadtmädchen im Vergleich zu den Landmädchen früher menstruieren. Auch die Knaben aus den Städten reifen früher als die Knaben vom Lande (WESTLING).

In Finnland hat HEINRICIUS sein Material in solche Mädchen eingeteilt, welche in Helsinki geboren sind und dort wohnen, sowie in solche, die auf dem Lande geboren und nach Helsinki umgezogen sind. Die von anderen Städten zugezogenen Mädchen hat er von den vom Lande gekommenen nicht unterschieden. HEINRICIUS konnte keinen Unterschied zwischen diesen beiden Gruppen wahrnehmen.

In meinem eigenen Material verteilt das Menarchealter nach dem Wohnort sich folgendermassen:

	Anzahl	Menarchealter
Stadtmädchen	1713	$14{,}08 \pm 0{,}03$ Jahre
Landmädchen	1329	$14{,}46 \pm 0{,}04$ „

Die Differenz ist signifikant, $0{,}38 \pm 0{,}05$ Jahre, und demnach bekommen in Finnland die in der Stadt wohnenden Mädchen ihre erste Menstruation früher als die auf dem Lande wohnenden. Wenn man das Material nach dem Geburtsort in auf dem Lande und in der Stadt geborene einteilt, ist der Unterschied noch deutlicher:

	Anzahl	Menarchealter
In der Stadt geborene	1463	$14{,}01 \pm 0{,}04$ Jahre
Auf dem Lande geborene	1483	$14{,}47 \pm 0{,}04$ „

Die Differenz ist $0{,}46 \pm 0{,}05$ Jahre. Den Unterschied zwischen den in der Stadt geborenen und in der Stadt wohnenden sowie zwischen den auf dem Lande geborenen und auf dem Lande wohnenden Mädchen ist nicht signifikant. Von den auf dem Lande geborenen hatten in meinem Material verhältnismässig mehr ihre erste Menstruation nach dem Alter von 17,5 Jahren bekommen, nämlich 40 Fälle, während weiterhin auf den Lande nur 25 solche Mädchen wohnten. Im Alter von weniger als 11,6 Jahren hatten

von den auf dem Lande geborenen Mädchen 18 menstruiert, von den weiterhin dort wohnenden 13. Von den in der Stadt geborenen wiederum war nur bei 8 die erste Menstruation erst nach 17,5 Jahren eingetreten, bei den in der Stadt wohnenden war die entsprechende Zahl grösser, 24. Von denjenigen, welche ihre erste Mentruation früh, vor 11,6 Jahren bekommen hatten, waren 30 in der Stadt geboren und entsprechend wohnten in der Stadt 38. Demnach waren also vom Lande in die Stadt relativ mehr spät menstruierende Mädchen umgezogen, und darauf ist es zurückzuführen, dass bei der Berechnung nach dem Wohnort die Stadtmädchen ein verhältnismässig höheres Menarchealter haben.

Die Ursache für den Unterschied im Menarchealter der Stadt- und der Landmädchen ist sowohl in sozialen, die Nahrung betreffenden als auch klimatischen Umständen gesucht worden. Die in der Stadt wohnenden haben überhaupt bessere Lebensbedingungen, die Nahrung ist vielseitiger, proteinhaltiger, die hygienischen Verhältnisse sind besser, und auch der Schulunterricht als eine gewisse geistige Reizung ist in Betracht zu ziehen. Desgleichen haben die anderen psychischen Anreize, wie der lebhaftere Lebenstakt, die Vergnügungen und der Umgang mit der Jugend anderen Geschlecht ihre Bedeutung (Schaeffer, Rossi Doria, Skerlj). Neurath teilt die Menschen in Kultur- und Naturtypen ein, und ist mit Kleinschmidt gleicher Meinung darüber, dass alles, was die geistige Reife des Kindes fördert, die ganze intensive Lebensweise, und alles, was geschlechtlich reizt, auch die körperliche Entwicklung fördert. Gewissermassen noch weiter geht in seiner Einteilung Mathes, welcher sagt: "Es wird meist gesagt, dass die äusseren Verhältnisse des Grossstadtlebens den frühen Eintritt der Geschlechtsreife bedingen. Wenn man grundsätzlich auf dem Standpunkt steht, dass in dem Widerstreit der Energien von Keimanlage und äusseren Einflüssen die ersteren immer die Oberhand behalten, wird man zur Erklärung, warum Grossstadtmädchen früher reifen, eher an die Momente denken müssen, die die Menschen eben in die Grossstadt treiben, die sie zu Grossstadtmenschen machen. In der Grossstadt treffen sich die Menschen aus allen Zonen, Angehörige der verschiedenen Nationen und Rassen, die Grossstadtbevölkerung ist eine Rasse für sich und so auch in Bezug auf die Zeit des Eintritts der Geschlechtsreife".

Schaeffer stellte fest, dass in je grösserer Stadt das Mädchen lebte, es umso früher seine Geschlechtsreife erreichte, und dass der Wohnort, nicht der Geburtsort ausschlaggebend das Menarchealter beeinflusste, obwohl andererseits die Umsiedelung in das Stadtleben an sich das Auftreten der ersten Menstruation verzögern konnte. Meine eigene Arbeit stützt Schaeffers Theorie nicht, weil in meinem Material nach dem Geburtsort der Unterschied im Menarchealter deutlicher war als bei Berechnung nach dem Wohnort. Lennér in Schweden hat ebenfalls festgestellt, dass der Unterschied durch den Wohnort bedingt war: Landgegend: Menarchealter $14{,}71 \pm 0{,}06$ Jahre, Stadt: $14{,}40 \pm 0{,}05$ Jahre. Lennér vermutet, dass der Unterschied zwischen Stadt und Land ständig kleiner werde, je enger die Landgegenden mit dem Wirkungskreis der Stadt verknüpft werden.

Als Kuriosität sei erwähnt, dass Krieger, dessen Arbeit in eine Zeit fiel, als dem Klima ausschlaggebende Bedeutung für das Menarchealter zugeschrieben wurde, vermutete, die Stadtmädchen reiften deshalb früher als die Landmädchen, weil die mittlere Temperatur in den Städten höher war als in den Landgegenden.

Wilson und Sutherland stellten keinen wesentlichen Unterschied zwischen dem Menarchealter der Schulmädchen auf dem Lande und der Schulmädchen in der Stadt fest, und Schreiner wiederum konstatierte, dass die Landbevölkerung fast früher reift als die Städter. Lintz und Markow machen eine Ausnahme, indem bei ihnen das Menarchealter der Stadtmädchen ca. ½ Jahr später liegt als bei den Landmädchen.

Es erscheint glaubhaft, dass die in Mathes' Theorie aufgeführten Momente sowie die Verschiedenheit der geistigen und körperlichen Reize und die verschiedenen hygienischen Verhältnisse die frühere Geschlechtsreife der Stadtmädchen im Vergleich zu den Landmädchen bedingen.

Auf Grund meiner Untersuchungen hat sich ergeben, dass *in Finnland bei den Stadtmädchen die erste Menstruation früher eintritt als bei den Landmädchen.* Der Unterschied ist beträchtlich, $0{,}38 \pm 0{,}05$ Jahre nach dem Wohnort und $0{,}46 \pm 0{,}05$ Jahre nach dem Geburtsort berechnet. Obwohl bei uns niemals früher in grösserem Umfange genauere Berechnungen angestellt worden sind, so ist es doch wahrscheinlich, dass auch in Finnland früher

ein grösserer Unterschied zwischen dem Menarchealter der Stadtmädchen und dem entsprechenden Alter der Landmädchen bestand als heutzutage.

Während unserer Kriege und nach denselben hat bei uns ein viel grösserer Prozentsatz der Bevölkerung seinen Wohnort gewechselt als normalerweise. Die Bevölkerung des abgetretenen Teils von Karjala verliess in ihrer Gesamtheit ihre früheren Gebiete und siedelte nach Stammfinnland um, desgleichen die Bewohner von Petsamo und von dem gepachteten Gebiete Porkkala. Die beiden letztgenannten stellen jedoch nur einen kleinen Teil der Evakuierten dar, von welchen die Karelier ca. ½ Million ausmachen. Die Bewohner der drei Städte von Karjala zogen in erster Linie wieder in Städte, während die Landbewohner im grossen und ganzen alle auch in ihren neuen Wohngegenden auf dem Lande blieben.

Im Zusammenhang mit meiner Arbeit habe ich auch zu klären versucht, welche Wirkung diese Umsiedelungen auf das Heranreifen der Mädchen zu Frauen gehabt hat. Wenn die Umsiedelung innerhalb der gleichen Provinz von einer Stadt in die andere stattgefunden hatte, oder von einer Landgegend der gleichen Provinz in die andere, so wurde dieser Fall nicht mitgenommen. Auf Grund meines Materials habe ich eine Tabelle zusammengestellt, aus welcher die Wirkung der Umsiedelungen auf das Menarchealter hervorgeht. Zum Vergleich habe ich daneben auch die entsprechenden Zahlen der nicht umgesiedelten Mädchen aufgeführt.

	Nicht umgesiedelt		Umgesiedelt			
	S	L	S–S	L–L	S–L	L–S
N:o	1	2	3	4	5	6
Anzahl	865	859	401	201	192	396
Menarchealter .	13,97	14,49	14,02	14,53	14,17	14,35
ε(M)	0,05	0,05	0,07	0,09	0,09	0,08

S = Stadtmädchen, L = Landmädchen, S — S = von Stadt zu Stadt, L — L = von einer Landgegend in die andere, S — L = von der Stadt aufs Land und L — S = vom Lande in die Stadt umgezogene Mädchen.

Zwischen dem Menarchealter der ständig in der Stadt und der ständig auf dem Lande wohnenden Mädchen besteht demnach wie auch sonst ein deutlicher Unterschied, 0,52 ± 0,06 Jahre. Der Umzug von einer Stadt in die andere hat das Menarchealter der Mädchen nicht beeinflusst, und ebensowenig die Umsiedelung von der Stadt aufs Land. In der Tabelle S. 50 ist die Differenz zwischen den Gruppen 1—2, 1—4, 1—6, 2—3, 2—5, 3—4, 3—6 und 4—5 signifikant. Es hat den Anschein, dass das Menarchealter dem nach dem Geburtsort berechneten entsprechenden Alter folgt, insofern als bei einem von der Stadt auf das Land umgezogenen Mädchen das Menarchealter demjenigen der Stadtmädchen im allgemeinen näher kommt als demjenigen der Landmädchen und entsprechend bei vom Lande in die Stadt umgesiedelten dem Menarchealter der Landbewohner. Es ist demnach wahrscheinlich, dass der Geburtsort die Zeit der Geschlechtsreife bestimmt und nicht der Wohnort.

Die erschütternde zwangsweise Umsiedelung der Karelier könnte auch auf eine Lebensfunktion wie das Auftreten der ersten Menstruation Einfluss gehabt haben. Diesen Umstand habe ich noch gesondert untersucht, indem ich die eigentlichen Evakuierten mit denjenigen verglich, welche innerhalb der gleichen Provinz umgezogen waren:

	Gleiche Provinz		Karelische Evakuierte			
	S–L	L–S	S–S	L–L	S–L	L–S
N:o	1	2	3	4	5	6
Anzahl	67	140	212	60	68	107
Menarchealter .	14,16	14,46	14,18	13,98	14,24	14,49
ε(M)	0,14	0,15	0,10	0,15	0,17	0,14

Der Unterschied zwischen den Gruppen 1 und 2 ist kein wirklicher Unterschied. Die Gruppe 1 ist zwar sehr klein, nur 67 Fälle, die Gruppe 2 allerdings grösser, 140 Fälle, aber die Streuung ist auch gross, es gehören Mädchen dazu, deren Menarchealter von 10,6 Jahren bis zu 20,5 Jahren schwankt. Die Differenzen zwischen der Gruppe 1 und den übrigen Gruppen sind keine wirklichen Unterschiede, dagegen sind die Differenzen zwischen der

Gruppe 2 zu Gruppe 3 und 5 signifikant. Die im Kreise der Evakuierten von einer Stadt in die andere umgezogenen verhielten sich zu den von Landgegend zu Landgegend umgesiedelten wie die Städter zu den Landbewohnern. Der Unterschied ist 0,41 ± 0,17 Jahre. Die von der Stadt aufs Land umgesiedelten verhalten sich zu den von einer Landgegend in die andere umgezogenen in gleicher Weise, die Differenz ist 0,51 ± 0,15 Jahre.

Im Vergleich zum Menarchealter der nicht Umgesiedelten scheint das entsprechende Alter bei den Kareliern nicht zu wachsen. Desgleichen ist das Verhältnis zwischen den zu den Evakuierten zählenden 447 Fällen und den nicht Evakuierten 549 Fällen gleichgeblieben.

	Nicht Evakuierte			
	S–L	L–L	S–S	L–S
Anzahl	188	106	64	191
Menarchealter	14,19	14,54	14,17	14,42
ε (M)	0,10	0,12	0,18	0,11

Der Unterschied zwischen L — L bei den Evakuierten und S — S bei den nicht Evakuierten ist nicht signifikant: 0,30 ± 0,18.

Schaeffer verglich im Jahre 1908 das Menarchealter bei in der Stadt geborenen und aufgewachsenen und vom Lande in die Stadt umgezogenen Mädchen miteinander und erhielt dabei einen sehr grossen Unterschied, nahezu 2 Jahre. Er war der Ansicht, dass am gewichtigsten für das Auftreten der ersten Menstruation der Ort sei, wo das Mädchen aufgewachsen ist, und nicht der Geburtsort. Da ich bei meiner Untersuchung mich nicht gesondert nach dem Alter der Mädchen bei der Änderung des Wohnortes erkundigt habe, kann ich nicht mit Sicherheit meine eigene Meinung aussprechen, wenn man aber die Resultate betrachtet, so erscheint es doch offenbar, dass der Geburtsort trotz allem ausschlaggebenden Einfluss auf den Verlauf der Entwicklung bei den Mädchen habe. Diese Vermutung wird meines Erachtens auch gestützt von der Feststellung, dass das Menarchealter der Mutter auf hereditärem Wege das Menarchealter der Töchter beeinflusst

(Lintz und Markow). Die gleichen Autoren stellen sogar die Einwirkung der Umgebung ganz in Abrede, indem sie die Erblichkeit als den wesentlichen Faktor ansehen, und sie sprechen die Ansicht aus, dass die Geschwister in gleichen Verhältnissen nicht deshalb ihre erste Menstruation im gleichen Alter bekommen, weil die äusseren Verhältnisse die gleichen sind, sondern weil sie die gleichen hereditären Eigenschaften haben. In meinem Material geben die bei der umgesiedelten Bevölkerung erhaltenen Resultete im Vergleich zu den bei den Mädchen von Stammfinnland berechneten Schaeffers Ansicht, dass die Umsiedelung den Reifungsprozess verzögernd beeinflusse, keine Stütze.

Aus meinen Untersuchungen geht hervor, dass die *in der Stadt geborenen und aufgewachsenen Mädchen ihre erste Menstruation früher bekommen als die auf dem Lande geborenen und aufgewachsenen Mädchen. Die Differenz ist 0,38 ± 0,05 Jahre.*

Es erscheint offenbar, dass der Geburtsort ausschlaggebend das Menarchealter beeinflusst. Es hat sich auch gezeigt, dass *die zwangsweise Umsiedelung, ungeachtet aller sowohl körperlicher als auch psychischer Strapazen die Entwicklung der karelischen Mädchen zur Geschlechtsreife nicht verzögernd beeinflusst hat.*

6. Einige spezielle Beobachtungen über die Menarche.

a. Die Menarche und die Regelmässigkeit der Menstruation.

Die Menarche braucht kein Zeichen dafür zu sein, dass das Mädchen geschlechtsreif ist, denn oft ist die erste Blutung nicht die Folge davon, dass eine Eizelle sich vom Ovarium abgelöst hat, sondern davon, dass die Ovarien ihre Tätigkeit beginnen, und dass sich Follikelhormon im Organismus zu bilden anfängt (Fraenkel). Auch die auf den ersten Blutfluss folgenden Blutungen sind nicht immer Folgen der Ovulation, und deshalb tritt die Menstruation auch am Anfang nicht immer unbedingt regelmässig auf. Nach der Menarche kann es sogar ein Jahr lang dauern, bis die nächste Menstruation eintritt (Rydberg), meistens ist sie jedoch schon

nach einem halben Jahr regelmässig (Scipiades, Engle, Görtz). Unregelmässigkeit am Anfang der Menstruation beruht jedoch nicht immer auf unregelmässiger Tätigkeit der Ovarien, sondern sie kann auch auf eine Krankheit zurückzuführen sein, z.B. bei anämischen Mädchen ist nach der Menarche eine lange, Jahre dauernde Pause oder sehr spärliche Blutung beobachtet worden (Schreiner). Auch die in der Kindheit durchgemachten Krankheiten, welche schon im Pubertätsalter gänzlich geheilt sind, können den Organismus so schwer geschädigt haben, dass die Menstruation unregelmässig ist und die Entwicklung verzögert. Dies betrifft nicht nur die schweren Infektionskrankheiten wie Tuberkulose und Lues, sondern auch sehr wesentlich die Rachitis (Görtz). Dworzak hat bei seinem 2400 Frauen umfassenden Material festgestellt, dass bei denjenigen, welche deutliche Spuren von geheilter Rachitis haben, die Menarche 2—4 Jahre später auftritt als bei den nicht rachitischen. Bei Frauen mit verengtem Becken hat Malmio ein Menarchealter von 16,21 ± 0,10 Jahren festgestellt, während wiederum bei den Frauen mit normalem Becken die entsprechende Zahl 15,96 ± 0,03 Jahre war. Nach Kaartinen wiederum war bei Mädchen, die im Pubertätsalter Struma hatten, das Menarchealter niedriger als bei den strumafreien.

Im meinen Karten war in 2491 Fällen angegeben worden, dass die Menstruation gleich von Anfang an regelmässig gewesen war sowie in 319 Fällen unregelmässig.

	Anzahl	Menarchealter
Regelmässig	2491	14,21 ± 0,03 Jahre
Unregelmässig	319	14,47 ± 0,09 „

Der Unterschied zwischen diesen beiden Zahlen ist eine wirkliche Differenz, 0,26 ± 0,07. In 2491 Fällen hatte die Menstruation früher begonnen und war regelmässig weitergegangen, in 312 Fällen wiederum ist die Tätigkeit der Ovarien und der anderen an der Menstruation beteiligten Organe weniger regelmässig gewesen und begann später als bei den erstgenannten. Engle und Shelesnyak sind der Ansicht, dass die Unregelmässigkeit der Menstruation und das Menarchealter keine Beziehung zueinander

hätten. Meine Untersuchung zeigt jedoch, dass dies der Fall ist, denn hiernach war bei einer Frau, bei welcher die Menstruation nach der Menarche regelmässig gewesen war, die Menarche früher eingetreten als bei solchen, deren Menstruation nach der ersten Blutung unregelmässig war. Der Unterschied ist ausserdem ziemlich gross.

Aus dem Obigen könnte man den Schluss ziehen, dass der Organismus derjeniger Mädchen, bei denen die Menstruation sofort nach der Menarche regelmässig wurde, trotz des niedrigeren Alters besser entwickelt, fertiger ist als bei den anderen, welche ihre erste Menstruation später bekommen, und deren Organismus auch danach schwächer arbeitet.

b. Die Menarche und die Menstruationsbeschwerden.

Das Verhältnis der Menarche und der Menstruationsbeschwerden ist umgekehrt wie das obige:

	Anzahl	Menarchealter
Keine Schwerzen	990	$14{,}21 \pm 0{,}05$ Jahre
Schwerzen	847	$14{,}04 \pm 0{,}05$ „

Der Unterschied zwischen den erhaltenen Zahlen ist eine wirkliche Differenz, $0{,}17 \pm 0{,}07$. Die obigen Zahlen zeigen, dass bei den Frauen, welche schmerzhafte Menstruation haben, die Menarche früher eingetreten ist als bei solchen, die keine Schmerzen haben. Ein Mädchen, dass seine erste Menstruation früher bekam, hatte regelmässigere Blutungen, aber schmerzhaftere als ein Mädchen, bei welchem die Menstruation später begann und unregelmässig war.

Die Schmerzen im Zusammenhang mit der Menstruation beruhen im allgemeinen auf der Konstitution des Uterus (Rydberg), die Regelmässigkeit der Blutung wiederum auf der Hormonsekretion. Man sollte annehmen, dass der Organismus eines später menstruierenden Mädchens besser auf seine Aufgabe vorbereitet wäre, aber Vara hat gezeigt, dass bei den jungen Erstgebärenden die Menarche früher eingetreten ist als bei den alten Erstgebärenden. Es ist festgestellt worden, dass die Ursache für das

späte Auftreten der Menarche oft späte Entwicklung der Geschlechtsorgane, wie Infantilismus und Hypoplasie ist (Malmio, Vehaskari, Aschan), und Rydberg hält es für möglich, dass der juvenile Dysmenorrhöetypus bei einem gewissen Konstitutionstypus, d.h. bei den asthenischen Frauen häufiger vorkäme, aber seines Erachtens besteht zwischen den verschiedenen Konstitutionstypen und der schmerzhaften Menstruation doch keine deutliche Beziehung. Die meisten Frauen haben zum mindesten geringe Beschwerden während der Menstruation oder am Anfang derselben. Nur eine kleine Anzahl, 16 %, fühlt keinerlei Störungen, und noch weniger, 7 %, fühlt sich geradezu besser und ist tatkräftiger als gewöhnlich (Tobler). Es wird vermutet, dass die Menstruationsschmerzen und das Übelsein zum Teil durch toxisch wirkende Stoffe bedingt sind, welche sich im Organismus angesammelt haben (Fraenkel), aber bei den jungen Mädchen dürfte die Ursache für die Schmerzen meistens im Körperbau zu suchen sein. Nach dem Konstitutionstypus ist bei den Pyknikern die frühe Menarche, im Alter von 10—12 Jahren, gewöhnlicher als bei den Leptosomen. Die Leptosomen wiederum ihrerseits bekommen öfter als die Pykniker ihre Menarche spät, im Alter von 17—18 Jahren (Schreiner). Von allen Konstitutionstypen haben 50 % am Anfang Menstruationsbeschwerden, je mehr Zeit aber nach der Menarche vergeht, umso allgemeiner sind die Schmerzen beim infantilen Typus (Görtz).

c. *Die Menarche, die Körperlänge und das Gewicht.*

In meinem Material habe ich auch das Menarchealter der Mädchen von normalem, Über- und Untergewicht berechnet. Das Verhältnis zwischen Gewicht und Länge entnahm ich aus der "Längen- und Gewichtstabelle der finnischen Jugend im Schulalter", welche Vartia zusammengestellt hat.

	Anzahl	Menarchealter	
Normalgewicht	1456	14,27 ± 0,04	Jahre
Untergewicht	252	14,28 ± 0,09	„
Übergewicht	1292	14,22 ± 0,04	„

Die Unterschiede zwischen den so erhaltenen Zahlen sind keine wirklichen Differenzen. Mein Material enthält viele schon etwas ältere Frauen, bei denen sich das Verhältnis zwischen Gewicht und Länge im Laufe der Jahre schon verändert hat. Aus meinen Berechnungen geht demnach hervor, dass sich aus der Konstitution, in diesem Falle aus dem Verhältnis zwischen Länge und Gewicht, nach meinem Material nicht schliessen lässt, bei wem die Reife früher eingetreten ist, bei wem später.

Trotzdem ist das Menarchealter abhängig vom Längen- und Gewichtswachstum. Unter gleichaltrigen Mädchen sind diejenigen, welche ihre erste Menstruation schon gehabt haben, im allgemeinen sowohl länger als auch schwerer als die noch nicht gereiften Mädchen (Stone und Barker). Dass die Korrelation zwischen Gewicht und Menarche grösser ist als zwischen Länge und Menarche (Peller und Zimmermann), hat auch Salo gezeigt, nach dessen Material 3,6 % von den Mädchen die Menarche nach Erreichung des Maximums im Längenwachstum bekamen, 96,4 % hingegen erst nachdem sie das Maximum ihres Gewichts erreicht hatten. Die körperliche Entwicklung der Mittelschüler im Vergleich zu den Volksschülern ist auch in Finnland schneller (Tunkelo, Lojander). Die Schülerinnen der Mittelschulen sind zur Zeit ihrer Menarche oft noch körperlich unentwickelt, wie grosse Kinder, während dagegen bei den Volksschülern auch die sekundären Geschlechtsmerkmale sich schon gleichzeitig mit dem Auftreten der ersten Menstruation entwickeln (Schreiner). Auf die frühere Geschlechtsreife folgt schnellere geistige Reife, und deshalb sollte man auch das Alter des Kindes nicht nach den Kalenderjahren sondern nach der geistigen Entwicklung bestimmen, sowohl in der Schule als auch vor dem Gesetz (Stone, Schiötz, Johnston).

d. Das Verhalten zu den Leibesübungen und die Schulleistungen.

In unseren Schulen dürfen die Mädchen im allgemeinen selbst bestimmen, ob sie während der Menstruation an den Leibesübungen teilnehmen wollen. Um zu sehen, in welchem Masse die Menstruationsbeshwerden in den negativen Fällen beteiligt sind,

habe ich 654 Mädchen, welche während der Menstruation keinen Sport treiben, mit 121 solchen verglichen, die während der Menstruation an den Leibesübungen teilnehmen.

	Nimmt an den Leibesübungen nicht teil	Nimmt an den Leibesübungen teil	Zusammen
Schmerzen	a) 316	b) 36	352
Keine Schmerzen	c) 338	d) 85	423
Zusammen	654	121	775

Der aus dieser Tabelle berechnete Wert χ^2 nach der Formel

$$\chi^2 = \frac{(ad - bc)^2 \ (a + b + c + d)}{(a + b) \ (c + d) \ (a + c) \ (b + d)}$$ ist $\chi^2 = 14{,}20$. Die Zahl ist genügend gross um zu zeigen, dass ein offenbarer Unterschied im Verhalten der an Menstruationsschmerzen leidenden und der davon freien Mädchen zu den Leibesübungen besteht.

Es ist nachgewiesen worden, dass χ^2 eine ganz bestimmte Verteilung befolgt, und mit Hilfe dessen kann man schliessen, ob die in dem Material festgestellten Unterschiede durch zufällige Schwankungen bedingt oder signifikant sind.

Nach meiner Untersuchung hat es den Anschein, dass die Mädchen, welche schmerzhafte Menstruation haben, sich während derselben lieber von den Turnstunden zurückhalten, während wieder diejenigen, bei denen die Menstruation ohne fühlbarere Beschwerden verläuft, verhältnismässig öfter trotzdem an den Leibesübungen teilnehmen.

Die Wichtigkeit des Turnens und des Sports im Pubertätsalter wird allgemein hervorgehoben (Aron, Neurath). Der wachsende Organismus braucht auch im allgemeinen Bewegung und Muskelübungen, aber besonders während des kräftigen, sowohl geistigen als auch körperlichen Wachstums ist diese Bewegung wichtig. Der günstige Einfluss der Leibesübungen macht sich während der ganzen Pubertätszeit und noch danach bemerkbar, denn die Zunahme der Muskeltätigkeit und das Wachsen der Muskeln hat eine Bremswirkung auf den Reifungsprozess (Aron). Dank der

Leibesübungen werden die Gedanken des jungen Mädchens in gesunde Bahnen gelenkt, und ein regelmässig Sport treibendes Mädchen überwindet die Pubertätszeit leichter und schmerzloser als ein Mädchen, welches keinen Sport treibt. Clow und Sanderson empfahlen den Mädchen des von ihnen überwachten Internats, auch während der Menstruation zu turnen und Sport zu treiben, und stellten danach bei den meisten im Allgemeinzustand und Wohlbefinden eine günstige Veränderung fest. Dünzer stellte Nachfragen bei 4434 Sport treibenden Mädchen an und konstatierte, dass inbesondere auf die jüngeren Alterklassen die Körperbewegung vorteilhaft einwirkte, indem sie die Menstruationsschmerzen erleichterte. Albrecht wiederum hält die Zunahme der Menstruationsstörungen für eine Folge der geistigen und körperlichen Überanstrengung der Jugendlichen. Selbstverständlich sind auch übermässige Anstrengungen, z.B. Sportwettkämpfe, während der Menstruation nicht zu empfehlen.

Meiner Untersuchung gemäss vermeidet ein Mädchen, dessen Menstruation schmerzhaft ist, häufiger das Turnen und den Sport während derselben als ein solches Mädchen, dessen Menstruation schmerzlos verläuft.

Da die Pubertätszeit vor der Menarche und danach für die meisten Mädchen eine Zeit voller Konflikte ist, wollte ich untersuchen, welchen Einfluss das Auftreten der Menarche auf die Schulleistungen hat. In unserem Lande bekommen die Mädchen ihre erste Menstruation durchschnittlich während der Mittelschulzeit, weshalb meine Untersuchung in dieser Beziehung sich nur auf die höheren Schulen beschränkt. Da in unseren höheren Schulen die verschiedenen Fächer verschiedenen Lehrern zugeteilt sind, wird bei der Beurteilung der Schülerinnen die Möglichkeit ausgeschieden, dass die Sympathien oder Antipathien der Lehrer die Gesamtbeurteilung beeinflussten. Das im Pubertätsalter stehende Mädchen ist oft eine schwer zu behandelnde Schülerin wegen ihrer Lebhaftigkeit und Ausfälligkeit, weshalb in den Schulen, wo jede Klasse nur einen Lehrer hat, das Zeugnis nicht als Masstab für die geistigen Fähigkeiten gelten kann, denn von einem schwer zu beherrschenden Schüler bekommt man leicht eine schlechtere Auffassung als seine Intelligenz voraussetzt (Stifter).

Auch sonst dürfte sich als Masstab für die Intelligenz nicht ausschliesslich das Schulzeugnis ansehen lassen, denn der Unterschied zwischen den Schulleistungen und den im späteren Leben erzielten Resultaten kann sogar sehr gross sein, je nachdem, ob die Neigungen der betreffenden Person in theoretischer oder praktischer Richtung gehen (JUST).

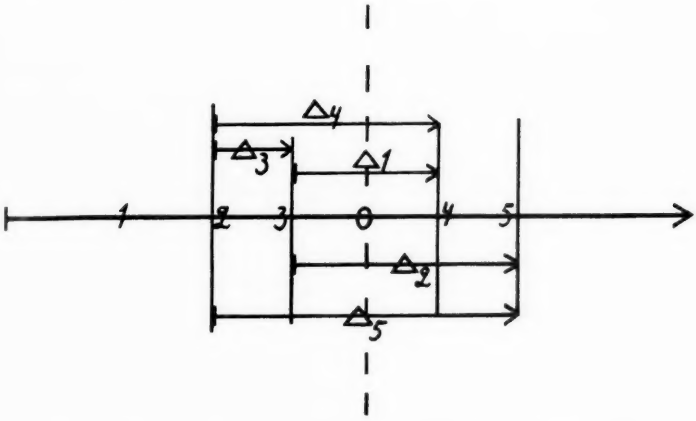
Bei 139 Mädchen, welche keine störenden Momente, wie Repitieren einer Klasse, durch den Krieg bedingte Unterbrechung oder schwerere Krankheiten hatten, habe ich den Mittelwert der wissenschaftlichen Fächer berechnet. Aus den auf Grund dieser Mittelwerte gezeichneten graphischen Kurven geht hervor, dass das Auftreten der Menarche weder Steigerung noch Sinken der Schulleistungen hervorruft. Auch bei solchen, welche ihre Menarche später als gewöhnlich bekamen, und die Serie der Noten vorher also lang ist, scheint kein Unterschied in den Leistungen vor und nach der Menarche festzustellen zu sein.

Nach den deutschen Autoren hat die Menarche Bedeutung für die Schulleistungen. So hat z.B. KING nachgewiesen, dass von gleichaltrigen Schülerinnen diejenigen, welche in der Pubertät standen und auch die schon menstruierenden, höhere Klassen erreichten und bessere Zeugnisse erhielten als die in der Praepubertät stehenden. STIFTER behauptet, es bestehe eine deutliche positive Korrelation zwischen der Körpergrösse, der Geschlechtsreife und den geistigen Leistungen.

Um genauer den Einfluss der Menarche selbst zu untersuchen, berechnete ich für alle 139 eine gemeinsame Tabelle, in welcher der Zeitpunkt der Menarche als O-Punkt angenommen wird. Mit + oder — ist darin das Steigen oder Sinken des Mittelwertes angegeben. Diese Zahlen verglich ich in der Gegend des Menarchealters miteinander. Um störende Momente, z.B. Krankheiten, möglichst zu vermeiden, nahm ich nur ganz naheliegende Werte zum Vergleich. Figur Nr. 3.

Ich verglich die Werte von 3 zu 4 = $\triangle_1$ = — 0,046 ± 0,031
3 zu 5 = $\triangle_2$ = — 0,026 ± 0,043
2 zu 3 = $\triangle_3$ = — 0,007 ± 0,034
2 zu 4 = $\triangle_4$ = — 0,083 ± 0,039
2 zu 5 = $\triangle_5$ = — 0,112 ± 0,044

Figur Nr. 3.



In denjenigen Fällen, wo der Auftritt der Menarche mit dem Zeugnis zusammenfiel, liess ich dasselbe als O-Wert weg. Alle fünf Vergleichszahlen bekamen einen negativen Wert, und die Zahlen sind sehr klein. Wie man sieht, besteht zwischen den Mittelwerten der Noten kurz vor der Menarche und kurz danach kein Unterschied, die Zahl ist ja nur $-0{,}046 \pm 0{,}031$, oder nahezu 5 Prozent der Note. Das gleiche Resultat bekommt man auch in den folgenden Fallen, erst $\triangle_4$, das Verhältnis des ca. ½ Jahr vor der Menarche erhaltenen Zeugnisses zu dem ca. ¼ Jahr danach erhaltenen ist etwas grösser, $-0{,}083 \pm 0{,}039$; und $\triangle_5$, die Differenz zwischen dem ca. ½ Jahr vor der Menarche erhaltenen Zeugnis und dem ca. ½ Jahr danach erhaltenen ist am grössten: $-0{,}112 \pm 0{,}044$. Demnach kann man vielleicht eine kleine Tendenz zum Sinken der Noten nach der Menarche faststellen. Zum Vergleich zeichnete ich die Kurven der Mittelwerte aus den Zeugnissen von 13 Knaben der Oberklassen. Da WESTLING auf Grund seines in Finnland gesammelten Materials festgestellt hat, dass bei den Knaben die erste Ejakulation durchschnittlich im Alter von 14.8 Jahren auftritt, so hätten auch die Knaben, von welchen ich den Mittelwert der Schulleistungen berechnete, dieses Alter schon überschnitten, und mögliche Veränderungen in den geistigen Leistungen wären sichtbar geworden. Die Kurven an sich weichen von den entsprechenden Kurven der Mädchen nicht wesentlich ab.

Der Schulbesuch bewirkt als ein geistiger Anreiz schnellere geistige Entwicklung, und gerade diese auf das Gehirn einwirkende Arbeit regt die endokrinen Drüsen zur Tätigkeit an, beschleunigt den Reifungsprozess, und die Folge davon ist, dass die Schülerinnen der höheren Schulen im Vergleich zu den anderen Mädchen früher ihre Menarche bekommen (ENGELMANN, KLEINSCHMIDT). Die Schülerinnen, welche während der Pubertätszeit nicht einmal zu mittelmässigen Leistungen fähig waren, können nach dem Auftreten der Menarche überraschend gute geistige Fähigkeiten zeigen. Hier sind gewisse Hemmungen vorhanden gewesen, welche die Menarche aufgehoben hat (MIROW).

Bei meiner Untersuchung darüber, ob die Menarche die geistigen Leistungen der Schülerin beeinflusst, bin ich zu einem negativen Resultat gekommen. Die auf Grund der Mittelwerte der wissenschaftlichen Fächer aus den Zeugnissen von 139 Mädchen gezeichneten graphischen Kurven zeigen weder deutliche Steigerung noch Sinken vor und nach der Menarche. Ebensowenig lässt sich aus den zusammengerechneten Noten dieser Mädchen eine Veränderung in steigender oder fallender Richtung zu beiden Seiten der Menarche wahrnehmen. Die Leistungen der Knaben aus den gleichen Klassen weichen von den Leistungen der Mädchen nicht wesentlich ab.

Aus dem Obigen geht hervor, dass das Alter der regelmässig menstruierenden Mädchen beim Eintritt der Menarche niedriger war als bei den unregelmässig menstruierenden. Die Differenz war 0,26 ± 0,07.

Diejenigen Mädchen, welche schmerzhafte Menstruation hatten, bekamen ihre Menarche früher als diejenigen, deren Menstruation schmerzlos war. Die Differenz war 0,17 ± 0,07.

Nach meinem Material lässt sich aus der Konstitution, in diesem Falle aus dem Verhältnis zwischen Länge und Gewicht nicht schliessen, bei wem die Menarche früher auftritt und bei wem später.

Die Mädchen, welche schmerzhafte Menstruation haben, vermeiden die Leibesübungen während derselben häufiger als diejenigen, deren Menstruation störungsfrei verläuft. Auf die Schulleistungen scheint das Auftreten der Menarche meinem Material gemäss keinen Einfluss zu haben.

7. Das Auftreten der Menarche in den verschiedenen Kalendermonaten.

In unserem Lande sind die Temperaturen der verschiedenen Jahreszeiten sehr unterschiedlich, der Winter ist kalt und sonnenlos, der Sommer, wennschon kurz, so doch warm und die Sonnenstrahlung stark. Deshalb ist die Beschaffenheit und der Vitamingehalt der Nahrung in den verschiedenen Kalendermonaten sehr ungleich. Dies wirkt u.a. auf den Fettgehalt der Muttermilch, welche in den Frühjahrsmonaten kleiner als in den Herbstmonaten ist (SALMI). Im Hinblick auf alle diese Umstände wollte ich untersuchen, ob diese Momente Einfluss auf den Reifungsprozess haben. Ich teilte zu diesem Zweck mein Material in Gruppen ein, je nachdem, in welchem Kalendermonat die Menarche aufgetreten war.

In meinem Material waren die Mädchen in den verschiedenen Monaten ungefähr im gleichen Verhältnis geboren, wie es auch nach den statistischen Feststellungen geschieht, nämlich so, dass im Oktober und November am wenigsten geboren waren, sowie im März und Mai am meisten. Gleich am Anfang meiner Berechnungen stellte ich fest, dass die Menarche bei vielen im gleichen Kalendermonat begonnen hatte, in welchem sie geboren waren, und deshalb führte ich eine Berechnung besonders im Hinblick darauf gesondert aus. In meinem Material hatten 5052 Mädchen genau den Monat der Menarche angegeben, 513 auch den Tag. Diejenigen Fälle, bei welchen die Angabe ungenauer gemacht worden war, wie "im Herbst", "im Sommer" usw., liess ich ausser acht.

Die Tabelle Nr. 2, Spalte 2, zeigt, wie zahlreich in jedem einzelnen Monat die Menarche aufgetreten war. Am häufigsten war dies im Juni vorgekommen, 616 Fälle, danach kommt der Januar mit 568 Fällen, dann der September mit 540. Am kleinsten war die Anzahl der Menarchefälle im November, 287 Fälle. Im Monat der Geburt war die Menarche bei 604 Mädchen aufgetreten, diese Fälle waren aber nicht gleichmässig auf alle Monate verteilt, Tabelle Nr. 2, Spalte 3, sondern die Anzahl schwankte von 81 bis 21. Ich hielt es für möglich, dass diese Fälle die ersteren Zahlen beeinflussen könnten, und liess sie deshalb von der Anzahl der auf die verschiedenen Kalendermonate verteilten Fälle weg, Spalte 4. Auf die Reihenfolge der Monate wirkte diese Ver-

Tabelle Nr. 2.

MENARCHEMONAT

MONAT	ANZAHL	IM GEBURTSMONAT	GEBURTSMONAT AUSGESCHIEDEN	TAGE AUSGLEICH	% P	ε_P	% q	ε_q
I	568	62	506	490	11,2	0,48	27,0	0,67
II	290	44	246	264	6,0	0,37		
III	437	53	384	372	8,5	0,42		
IV	439	54	385	385	8,8	0,43	24,1	0,65
V	367	55	312	302	6,9	0,39		
VI	616	57	559	559	12,7	0,51		
VII	419	51	368	356	8,1	0,41	26,9	0,67
VIII	372	39	273	264	6,0	0,37		
IX	540	81	459	459	10,5	0,46		
X	296	48	248	240	5,5	0,35	22,0	0,13
XI	287	21	266	266	6,1	0,37		
XII	481	39	442	428	9,7	0,45		
ZUS.	5052	604	4448	4385	100			

MENARCHE IM GEBURTSMONAT

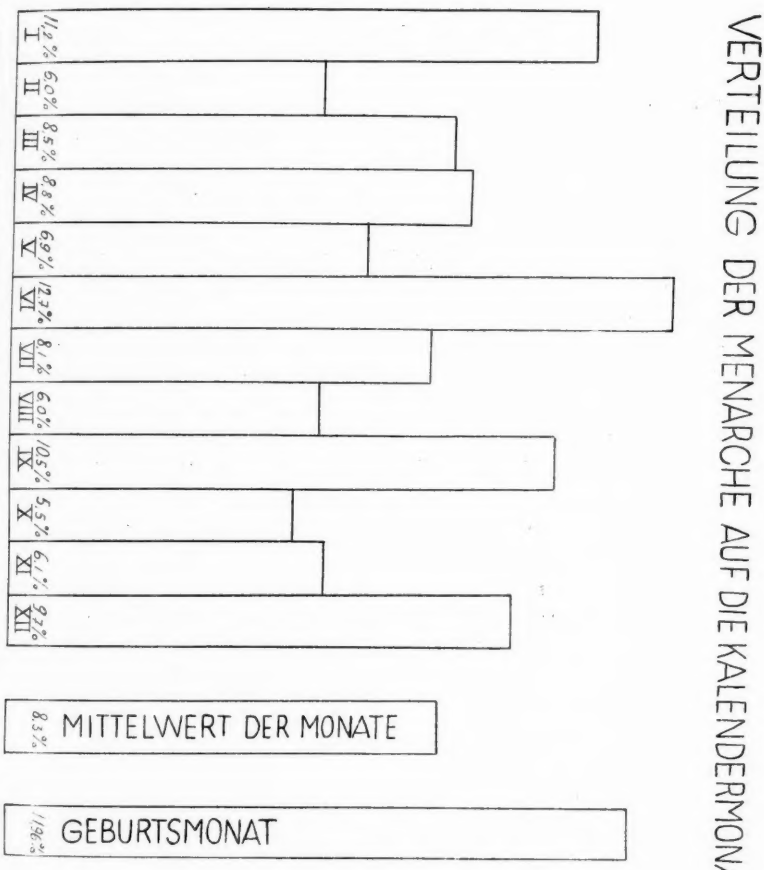
$$P = 11{,}96\ \% \qquad \varepsilon(P) = \sqrt{\frac{12 \cdot 88}{5052}} = 0{,}46 = 11{,}96 \pm 0{,}46\ \%$$

minderung nur insofern, als der an letzter Stelle gewesene November an die 10. Stelle rückte. Da die 31-tägigen Monate in Bezug auf die kürzeren in sog. günstigerer Stellung waren, nahm ich im Hinblick darauf einen Ausgleich vor:

$$\frac{30}{31} ; \frac{30}{28}$$

und danach, Spalte 5, blieb die Reihenfolge der Monate, mit vielen Menstruationsfällen unverändert, nur der November und der Februar bekamen "günstigere" Stellung. Aus den so erhaltenen Werten berechnete ich die Prozentzahlen. Aus den so durch

Figur Nr. 4.



Ausgleich für die verschiedenen Monate erhaltenen Prozentzahlen der Menarchefälle zeichnete ich eine Säulentabelle, (Figur Nr. 4) aus welcher hervorgeht, wie zahlreich die Fälle in jedem Monat vertreten sind, und wie sie sich zueinander verhalten. Der Februar und der August sind gleichgross, 6 %, desgleichen steht der November ungefähr auf dem gleichen Niveau, 6,1 %, und der

Mai mit 6,9 %. Zwischen diesen Zahlen besteht in meinen Berechnungen kein tatsächlicher Unterschied. Desgleichen ist der Unterschied zwischen den drei erstgenannten und dem kleinsten Monat, dem Oktober, 5,5 %, nicht signifikant. Die Differenz zwischen der Prozentzahl des Monats Mai und der entsprechenden Zahl des Oktober hingegen ist wirklich ($1{,}4 \pm 0{,}52$). Zwischen den folgenden Grösseklassen, März, April und Juli, besteht auch kein tatsächlicher Unterschied, aber die Differenz zwischen jeden derselben und den erstgenannten ist signifikant. Der Unterschied von März und April im Vergleich zum Dezember ist ebenfalls nicht wirklich. Die Differenz zwischen der Prozentzahl des Monats Januar und der Prozentzahl aller anderen Monate mit Ausnahme des Septembers ist signifikant, aber der Unterschied zwischen September und Dezember ist zu klein, um eine tatsächliche Differenz zu sein. Die Differenzen der Prozentzahlen aller anderen Monate sind signifikant. Neben die den Zahlen der Monate entsprechenden Säulen zeichnete ich eine dem Mittelwert der Monate entsprechende Säule, und neben diese wiederum eine dritte, welche die Anzahl der im Geburtsmonat aufgetretenen Menarchefälle angibt. Wenn man die Prozentzahl der im Geburtsmonat aufgetretenen Menarchefälle, $11{,}96 \pm 0{,}46$ mit dem Mittelwert der Prozentzahlen der verschiedenen Kalendermonate, $8{,}33 \pm 0{,}41$, vergleicht so ist die Differenz $= 3{,}63 \pm 0{,}62$. Nach meinem Material haben die Mädchen Neigung, ihre Menarche eben im Geburtsmonat zu bekommen.

Auch die Jahreszeiten verglich ich miteinander und das Resultat war folgendes:

Jahreszeit		Anzahl	%	ε (p)
III — IV — V	= Frühling ...	1059	24,1	0,65
VI — VII — VIII	= Sommer	1179	26,9	0,67
IX — X — XI	= Herbst	965	22,0	0,63
XII — I — II	= Winter	1182	27,0	0,67

Von den Prozentzahlen der Jahreszeiten ist die Differenz zwischen Winter und Sommer nicht signifikant, $0{,}1 \pm 0{,}94$, dahingegen ist dies zwischen den anderen Jahreszeiten der Fall. Die Prozentzahl des Winters ist am grössten, aber in diese Jahres-

zeit fallen auch zwei hochfrequente Monate, Dezember und Januar, und der Juni seinerseits erhöht wieder die entsprechende Zahl des Sommers. Die Frühjahrsmonate sind verhältnismässig gleich gross, aber zum Herbst gehört sowohl der grosse September als auch zwei kleine Monate, Oktober und November, und diese beiden letzteren drücken die Prozentzahl des Herbstes so weit herab, dass sie von den Zahlen der Jahreszeiten die kleinste ist.

Lennér hat in Schweden das Menarchealter der in den verschiedenen Monaten geborenen Mädchen berechnet, weil es möglich sein könnte, dass klimatische Momente, wie die Menge des Sonnenlichtes und der verschiedene Vitamingehalt der Nahrung, schon von Geburt an, eigentlich schon von früherer Zeit, Einfluss auf die Reifung des Mädchens hätten. Das Resultat war negativ.

Guggisberg hat durch Tierversuche gezeigt, dass verschiedene Vitamine grosse Bedeutung für das Menarchealter haben. Wenn die Nahrung sehr vitaminarm war, kam die Ovulation verspätet, ja sogar konnte Sterilität die Folge sein. Das C-Vitamin wird im allgemeinen als wichtig erachtet, desgleichen das E-Vitamin, aber auch A-Vitaminmangel verzögert die Reifung. Überreichliche Zufuhr von C-Vitamin kann auch schädlich wirken, wie Fontana gezeigt hat. Viele Sachverständige behaupten, dass reichlicher Fleischgenuss den Beginn der Menstruation fördere (Schaeffer, Guggisberg, Neurath). In unserem Lande dürfte Fleisch im allgemeinen reichlich gegessen werden, am meisten im Herbst und Winter, am wenigsten im Sommer.

Yamasaki hat schon im Jahre 1909 berechnet, dass in Japan die Mädchen ihre erste Menstruation meistens im Frühjahr bekommen, nach Scipiades war in Ungarn wiederum der August der gewöhnlichste Monat, und in New York trifft die Menarche selten im Frühling ein, nur in 18 % der Fälle, im Winter hingegen bei 29,6 % (Engle und Shelesnyak). Nach Breipohl kommt die Menarche "besonders in den Monaten Oktober bis Januar". Peller und Zimmermann untersuchten diese Erscheinung an einem Material von 1200 Mädchen, wobei die obere Altersgrenze 15 Jahre 3 Monate war. Auch nach ihrer Untersuchung verteilte das Vorkommen der Menarche sich nicht gleichmässig auf alle Kalendermonate, und die warme Jahreszeit war für das Auftreten der ersten Menstruation nicht gewöhnlicher als die übrigen

Jahreszeiten. Dahingegen war das Alter des Mädchens von Bedeutung, denn je älter es bei Beginn der Menstruation war, umso öfter traf dies im Herbst ein, je jünger wiederum, umso ungleichmässiger trat sie in den verschiedenen Monaten auf. Am gleichmässigsten verteilten sich die Fälle bei den 13—14-Jährigen. Der Geburtsmonat nahm nach ihrem Material keine Sonderstellung ein. Indem sie sich darauf beriefen, dass die Menarche bei denjenigen Mädchen, welche sie in jungem Alter bekommen, unkonsequent in den verschiedenen Kalendermonaten auftritt, vermuteten Peller und Zimmermann, dass psychische Faktoren ausschlaggebenden Anteil am Menarchealter haben könnten. Diese Annahme wurde ihres Erachtens auch noch dadurch gestützt, dass der Auftritt der Menarche während des Krieges langsamer wurde, obwohl an diesen Fällen aus der Kriegszeit auch der Hunger beteiligt sein konnte.

In meinem eigenen Material war die Verteilung auch auf die verschiedenen Kalendermonate nicht regelmässig. Der Unterschied zwischen dem grössten Monat, dem Juni, und dem kleinsten, dem Oktober, ist beträchtlich, $7{,}2 \pm 0{,}61$. Gerade im Juni, Januar, September und Dezember scheinen die Menarchefälle besonders zahlreich vorzukommen. Hieran könnten die Schulsemester ihren Anteil haben. Im September beginnt das neue Schuljahr, und das Bewusstsein neuer Pflichten könnte das Mädchen gerade in dieser Zeit zur Reife bringen. Im Dezember wiederum könnte möglicherweise das abschliessende Quartal und die bevorstehenden, ungefähr einen Monat dauernden Ferien psychisch auf das im Pubertätsstadium stehende Mädchen Einfluss haben, vielleicht auch trägt die Erwartung des Weihnachtsfestes ihren Teil bei. Der Januar könnte zum Teil eine Fortsetzung des Dezembers sein, und der Beginn des neuen Schulhalbjahrs hat ebenfalls seine Wirkung. Im Juni wiederum schliesst das Schuljahr ab, und die Erwartung der dreimonatlichen Ferien könnte befreiend auf den Organismus und seine Funktionen einwirken. Die relativ niedrige Anzahl von Menarchefällen im Mai stützt meines Erachtens diese Vermutung, denn im Mai gibt es noch zahlreiche Probearbeiten und dieser Monat ist auch sonst für die Schuljugend anstrengend.

Der Vitamingehalt der Nahrung ist in unserem Lande in den verschiedenen Jahreszeiten sehr unterschiedlich. Im Juni gibt es eigentliche Gemüse noch nicht sehr reichlich, aber die Milch dagegen ist dann schon vitaminreich. In Bezug auf den September könnte der Vitaminreichtum wohl Einfluss haben, aber wenn dieser Umstand sehr entscheidende Wirkung hätte, so wäre es eigentümlich, dass im Oktober, wo die Nahrung in dieser Beziehung noch vollen Gehalt hat, am wenigsten Menarchefälle vorkommen. Der Dezember und der Januar sind ebenfalls ungünstige Monate sowohl in Bezug auf das Sonnenlicht als auch auf die Vitamine. Im Frühjahr sind der März und der April dem Februar bedeutend voraus, obwohl die Nahrung gerade in diesen Monaten am vitaminärmsten ist. In dieser Zeit ist auch nach den Untersuchungen von Ritala der Haemoglobingehalt des Blutes am kleinsten. Auch nach dem Proteingehalt der Nahrung lässt sich schwer erklären, warum in den verschiedenen Monaten die Menarchefälle in so unterschiedlicher Anzahl auftreten. Im Herbst wird viel Fleisch gegessen, sowohl im September als auch in den folgenden Monaten, und doch ist der Unterschied zwischen diesen Monaten gross. Im Dezember und Januar könnte sich der Weihnachtsschinken auswirken, aber auch im Februar während der kältesten Zeit sind die Fleischgerichte sehr beliebt, und trotzdem kommen Menarchefälle wenig vor.

Ruotsalainen hat festgestellt, dass in unserem Lande die Jahreszeiten und klimatische Umstände sowohl auf das Längen- als auch das Gewichtswachstum der Kinder Einfluss haben. Der kräftigste Längenzuwachs erstreckt sich von Anfang Mai bis Anfang August, und der grösste Gewichtszuwachs geschieht in der Zeit von Anfang August bis zum November. Wenn der Gewichtszuwachs am grössten ist, tritt die Reifung ein. Vielleicht steht die grosse Anzahl der Menarchefälle im September gerade mit dem Gewichtszuwachs in Zusammenhang. Aber andererseits gehören der Oktober und November wieder zu den Monaten, in welchen die Menarche selten auftritt, und trotzdem ist während dieser Zeit der Anstieg der Gewichtskurve kräftig. In Bezug auf den Januar und Dezember lässt sich mit dieser Theorie keine Entscheidung treffen, denn die grösste Gewichtszunahme und darauf folgende Menarche dürften sich kaum bis in diese Monate

auswirken. Die Tatsache, dass nach meinem Material der Geburtsmonat des Mädchens sehr häufig auch der Monat der ersten Menstruation ist, $11{,}96 \pm 0{,}46$ %, stützt die Auffassung, dass psychische Momente bei der Auslösung des Reifungsprozesses grossen Anteil haben. Die Neigung zum Beginn der Menstruationstätigkeit im Geburtsmonat könnte man möglicherweise so erklären, dass das Mädchen dank der heutzutage allgemeinen Aufklärungsarbeit die erste Menstruation erwartet, und dann, wenn es ein bestimmtes Alter erreicht, die Menarche leicht gerade im gleichem Monat bekommt.

Die Menarche ist ein so umständlicher Prozess, dass kaum irgendwelche psychischen Momente allein imstande sein dürften, ihn in Gang zu bringen. Offenbar wird die Reife durch eine Kombination von zahlreichen Faktoren ausgelöst, aber die ganz bestimmte Verteilung der Menarche auf die Kalendermonate und die Häufigkeit der ersten Menstruation im Geburtsmonat stützen doch die Vermutung von der grossen Bedeutung der psychischen Momente.

Die Menarche verteilt sich nicht gleichmässig auf die verschiedenen Kalendermonate.

In den verschiedenen Jahreszeiten kommen Menarchefälle in verschiedener Frequenz vor.

Der Geburtsmonat ist offenbar eine gewöhnliche Zeit für das Auftreten der Menarche.

Von den Kalendermonaten ist im Juni die Prozentzahl der Menarchefälle am grössten, $12{,}7 \pm 0{,}51$ %, dann folgt der Januar mit $11{,}2 \pm 0{,}48$ %, an dritter Stelle kommt der September mit $10{,}5 \pm 0{,}46$ % und an vierter der Dezember mit $9{,}7 \pm 0{,}45$ %. Am kleisten ist die Prozentzahl der Menarchefälle im Oktober, $5{,}5 \pm 0{,}35$ %.

Den Jahreszeiten gemäss ist die Reihenfolge folgende: Winter $27{,}0 \pm 0{,}67$ %, Sommer $26{,}9 \pm 0{,}67$ %, Frühling $24{,}1 \pm 0{,}65$ %, Herbst $22{,}0 \pm 0{,}63$ %.

Die Prozentzahl des Geburtsmonats ist $11{,}96 \pm 0{,}46$.

Auf Grund der grossen Prozentzahl des Geburtsmonats hat es den Anschein, als ob die psychischen Momente grosse Bedeutung für das Auftreten der Menarche hätten.

V. Zusammenfassung.

Um das durchschnittliche Menarchealter der finnischen Frau bestimmen zu können, wurden in verschiedenen Gegenden Finnlands Angaben über diese Frage von 3075 Frauen und Mädchen gesammelt, und zwar von zwischen den Jahren 1923 und 1933 geborenen, von beiden Sprachengruppen, von finnisch- und schwedischsprechenden, aus verschiedenen sozialen Schichten stammenden, sowohl auf dem Lande als auch in der Stadt wohnenden. Als Resultat ergab sich, dass das durchschnittliche Menarchealter im Jahre 1950 in Finnland 14,25 ± 0,03 Jahre oder 14 Jahre 3 Monate ± 11 Tage war.

Im Jahre 1882 war das Menarchealter in Finnland auf 2,37 Jahre höher berechnet worden und im Jahre 1919 auf 1,70 ± 0,03 Jahre höher.

Die Kriegsjahre 1939—1944 scheinen keinen verspätenden Einfluss auf das Menarchealter der finnischen Mädchen gehabt zu haben.

Bei den finnischsprechenden Mädchen in Finnland ist das Menarchealter nach meinem Material höher als das entsprechende Alter bei den schwedischsprechenden Mädchen Finnlands. Bei den ersteren war es 14,28 ± 0,08 Jahre oder 14 Jahre 3 Monate 11 Tage ± 29 Tage und bei den letzteren 13,85 ± 0,09 Jahre oder 13 Jahre 10 Monate 6 Tage ± 32 Tage. Die Differenz war 0,43 ± 0,13 Jahre oder 5 Monate, 5 Tage ± 48 Tage.

Bei der Gruppierung nach den verschiedenen Stämmen ergab sich kein grösserer Unterschied zwischen den Gruppen.

Nach meinem Material tritt die Menarche bei den geistige Arbeit Verrichtenden oder ihren Töchtern früher auf als bei den körperliche Arbeit Verrichtenden oder ihren Töchtern. Bei den ersteren war dieses Alter 14,03 ± 0,04 Jahre oder 14 Jahre 11

Tage ± 14 Tage und bei den letzteren 14,39 ± 0,04 Jahre oder 14 Jahre 4 Monate 20 Tage ± 14 Tage. Die Differenz war 0,36 ± 0,05 Jahre oder 4 Monate 10 Tage ± 18 Tage.

Nach dem Wohnort gruppiert trat die Menarche bei den Städterinnen im Alter von 14,08 ± 0,03 Jahren und bei den Landmädchen im Alter von 14,46 ±0,04 Jahren auf, oder bei den ersteren im Alter von 14 Jahren 29 Tagen ±11 Tagen und bei den letzteren im Alter von 14 Jahren 5 Monaten 16 Tagen ± 14 Tagen. Bei den in der Stadt geborenen und in der Stadt wohnenden Mädchen trat die Menarche früher auf als bei den auf dem Lande geborenen und auf dem Lande wohnenden Mädchen. Es erscheint offenbar, dass der Wohnort Einfluss auf das Menarchealter hat.

Es hat sich aus den Untersuchungen ergeben, dass sie zwangsweise Umsiedelung die Entwicklung der karelischen Mädchen nicht verlangsamt hat.

Diejenigen Mädchen, bei welchen die Menstruation sofort nach der Menarche regelmässig wurde, waren beim Auftreten der ersten Menstruation durchschnittlich 14.21 ± 0,03 Jahre oder 14 Jahre 2 Monate 16 Tage ± 11 Tage alt und diejenigen, bei welchen die Menstruation nach der Menarche unregelmässig war, entsprechend 14,47 ± 0,09 oder 14 Jahre 5 Monate 19 Tage ± 32 Tage. Das Menarchealter der regelmässig menstruierenden Mädchen war demgemäss 0,26 ± 0,07 Jahre niedriger als dasjenige der unregelmässig menstruierenden.

Diejenigen Mädchen, die schmerzhafte Menstruation hatten, bekamen die Menarche früher als die Mädchen, deren Menstruation schmerzlos verlief. Nach meinem Material lässt sich aus der Konstitution, in diesem Falle aus dem Verhältnis zwischen Körperlänge und -gewicht, nicht feststellen, bei wem die Menarche früher aufgetreten ist und bei wem später.

Die Mädchen mit schmerzhafter Menstruation vermieden die Leibesübungen während des Monatsflusses öfter als diejenigen, welche keine Schmerzen hatten. Auf die Schulleistungen scheint das Auftreten der Menarche auf Grund meiner Arbeit keinen Einfluss zu haben.

Die Menarchefälle verteilen sich nicht gleichmässig auf die verschiedenen Kalendermonate und auch nicht auf die verschie-

denen Jahreszeiten. Im Juni kam die Menarche am häufigsten vor, darauf folgte der Januar, danach der September und Dezember. Der Geburtsmonat ist offenbar eine gewöhnliche Zeit für das Auftreten der ersten Menstruation, seine Prozentzahl war 11,96 ± 0,46. Nach meinem Material zu beurteilen scheinen die psychischen Faktoren grosse Bedeutung für das Menarchealter zu haben.

Summary.

In order to determine the average menarche age of Finnish women, information on this subject was collected from different parts of the country. The data concerned 3075 women and girls, born during the years 1923—1933, both Finnish- and Swedish-speaking people, belonging to different classes of society in the country and in towns, and the conclusion reached was that the average menarche age in Finland in 1950 was 14,25 ± 0,03 years, or 14 years 3 months ± 11 days. Since the year 1882 the menarche age in Finland has fallen by 2,37 years, and since 1919 by 1,70 ± 0,03 years.

The years of war (1939—1944) do not seem to have had any retarding influence on the menarche age of Finnish girls.

According to my material the menarche age of the Finnish-speaking girls was higher than the corresponding age of the Swedish-speaking girls. The menarche age of the Finnish-speaking girls was 14,28 ±0,08 years, or 14 years 3 months 11 days ± 29 days, and that of the Swedish-speaking girls 13,85 ± 0,09 years, or 13 years 10 months 6 days ± 32 days. The difference was 0,43 ± 0,13 years, or 5 months 5 days ± 48 days.

Grouped according to the different tribes the data revealed no great differences.

According to my material the menarche of intellectual workers and their daughters appered earlier than that of manual workers and their daughters. For the intellectual worker this age was 14,03 ± 0,04 years, or 14 years 11 days ± 14 days, and for the manual worker 14,39 ± 0,04 years, 14 years 4 months 20

days ± 14 days. The difference was 0,36 ± 0,05 years, or 4 months 10 days ± 18 days.

Grouped according to place of residence women living in towns attained the menarche at the age of 14,08 ± 0,03, and women in the country at the age of 14,46 ± 0,04, or the former at the age of 14 years 29 days ± 11 days, and the latter at the age of 14 years 5 months 16 days ± 14 days. The menarche of girls born and living in towns appeared earlier than that of girls born and living in the country.

It seems obvious that the place of residence has an influence on the appearance of the menarche. Compulsory change of place of residence proved to have no retarding influence on the development of puberty in Karelian girls.

The girls in whom menstruation became regular immediately after the appearance of the menarche were at the time of the menarche on an average 14,21 ± 0,03 years old, or 14 years 2 months 16 days ± 11 days, and those whose menstruation was irregular after the appearance of the menarche were correspondingly 14,47 ± 0,09 years old, or 14 years 5 months 19 days ± 32 days. Thus the age of girls whose menstruation was regular at the time of the menarche was 0,26 ± 0,07 years lower than that of girls with irregular menstruation.

Girls whose menstruation was painful had it earlier than girls whose menstruation was painless. According to my material it cannot be conclued from constitution, or from the relation of height to weight, in which of them the menarche will appear earlier, in which later. Girls whose menstruation was painful avoided gymnastics during menstruation more often than girls with painless menstruation. According to my material the appearance of the menarche seems to have no influence on school achievements.

The menarche is not distributed equally among the different calendar months. Most menarches fell in June, then in January, then in September and December. The month of birts is obviously a frequent time for the appearance of the menarche. The percentage in the month of birth was 11,96 ± 0,46. According to my material psychic factors seem to have a great influence on the age at which the menarche appears.

Résumé.

Afin de pouvoir fixer l'âge moyen de la ménarche chez la femme finlandaise, l'auteur a rassemblé dans toutes les régions du pays des renseignements sur ce sujet, consultant 3075 femmes et jeunes filles de langue finnoise ou suédoise, nées entre les années 1923—1933, représentant différentes classes sociales et habitant aussi bien la ville que la campagne. Il s'est révélé que l'âge moyen de la ménarche en Finlande en 1950 fur 14,25 ± 0,03 ans, soit 14 ans 3 mois ± 11 jours.

Depuis 1882, l'âge de la ménarche en Finlande a subi un abaissement de 2,37 ans, depuis 1919 1,70 ± 0,03 ans.

L'âge moyen de la ménarche chez les femmes finlandaises ne semble pas avoir subi une influence retardante du fait des années de guerre 1939—1944.

Selon le matériel que l'auteur a examiné, l'âge de la ménarche chez les jeunes filles finlandaises de langue finnoise a été plus élevé que chez les Finlandaises de langue suédoise. Chez les premières, il était de 14,28 ± 0,08 ans, soit 14 ans 3 moins ± 29 jours, chez les dernières, de 13,85 ± 0,09 ans, soit 13 ans 10 mois 6 jours ± 32 jours la différence étant donc 0,43 ± 0,13 ans, à savoir 5 mois 5 jours ± 48 jours.

Classés suivant les différentes tribus, les groupes ne présentent que de petites différences.

D'après le sujet d'étude, la ménarche a lieu plus tôt chez une femme faisant un travail intellectuel ou chez sa fille que chez celles qui font un travail corporel ou chez leurs filles. Chez le premier groupe, cet âge a été de 14,03 ± 0,04 ans, soit 14 ans 11 jours ± 14 jours, chez le dernier, de 14,39 ± 0,04 ans, soit 14 ans 4 mois 20 jours ± 14 jours. La différence a donc été de 0,36 ± 0,05 ans, à savoir 4 mois 10 jours ± 18 jours.

Selon le classement suivant le lieu d'habitation, la ménarche est apparue chez les citadines à l'âge de 14,08 ± 0,03 ans, chez les campagnardes à l'âge de 14,46 ± 0,04 ans, ce qui donne chez les premières l'âge de 14 ans 29 jours ± 11 jours, chez les dernières 14 ans 5 mois 16 jours ± 14 jours.

La ménarche chez les jeunes filles nées et habitant dans les villes s'est produite plus tôt que chez celles qui sont nées et

habitent à la campagne. Il est évident que le lieu d'habitation exerce une certaine influence sur l'âge de la ménarche.

Quant aux Caréliennes évacuées, il s'est révélé que l'émigration forcée n'a pas retardé leur développement physiologique de jeune fille en femme.

Les jeunes filles chez lesquelles la menstruation est devenue régulière dès la ménarche, ont eu au moment de celleci en moyenne 14,21 ± 0,03 ans, soit 14 ans 2 mois 16 jours ± 11 jours tandis que celles ayant des règles irrégulières après la ménarche ont eu 14,47 ± 0,09 ans, soit 14 ans 5 mois 19 jours ± 32 jours. L'âge de la ménarche des jeunes filles aux règles régulières fut donc de 0,26 ± 0,07 ans inférieur à celui des jeunes filles aux règles régulières.

Les jeunes filles à la menstruation douloureuse ont eu leur ménarche antérieurement à celles qui les ont subi sans douleurs. Le matériel ne montre pas quelles est l'influence qu'exerce la constitution, dans le cas présent le rapport entre la taille et le poids sur l'âge de la ménarche.

Les jeunes filles souffrant sensiblement de leur menstruation, ont plus fréquemment évité la gymnastique pendant cette période que celles dont les règles se sont passées sans douleurs. Quant aux résultats obtenus dans le travail scolaire, la ménarche n'y semble guère jouer de rôle, selon ce que prouve le présent matériel d'étude.

La ménarche ne se répartit pas d'une façon régulière entre les différents mois ni entre les saisons de l'année. Le mois de juin révèle le maximum en ménarches, janvier, septembre et décembre étant les suivants. Il paraît que le mois de naissance est bien souvent aussi le mois de ménarche et le pourcentage indique les chiffres de 11,96 ± 0,46. Les facteurs psychiques semblent avoir une grande importance pour l'âge de la ménarche.

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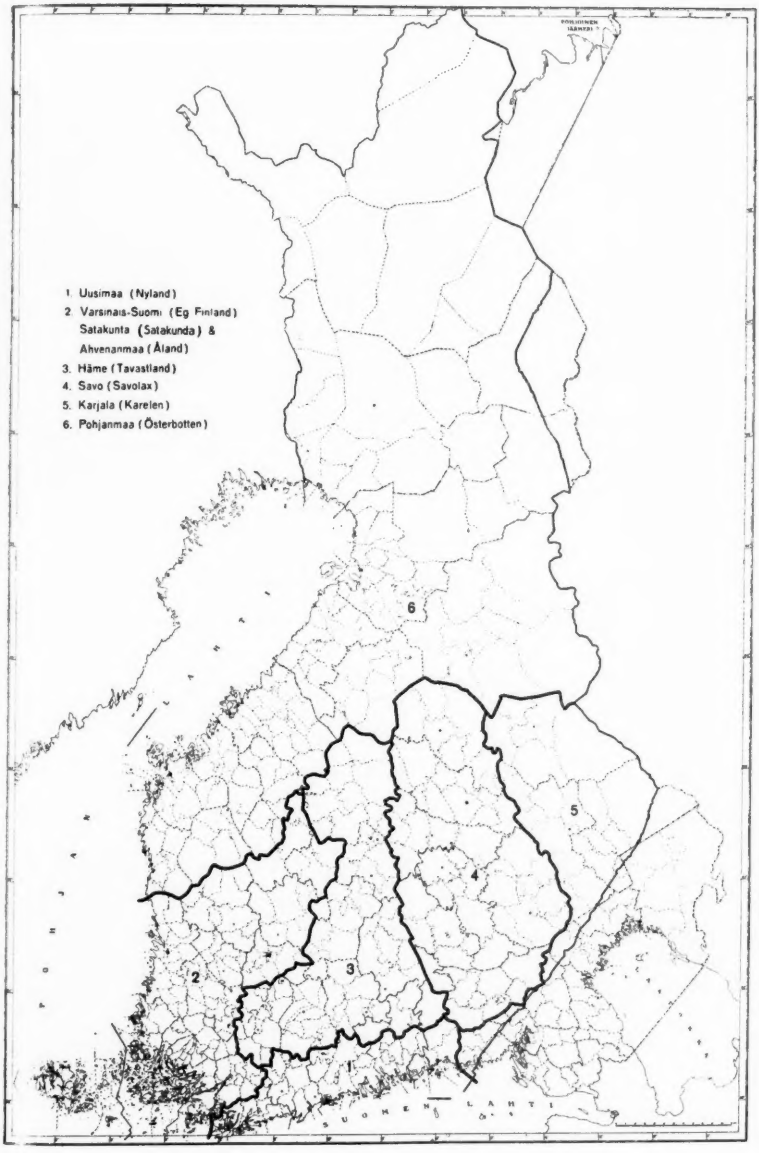
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5. Karjala (Karelen)
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POHJANLAHTI
SUOMENLAHTI

FROM THE SAMARITEN CHILDREN'S HOSPITAL, STOCKHOLM
(HEAD: PROFESSOR NILS MALMBERG)

COLONIC HYPERPERISTALSIS IN NEUROLABILE INFANTS

STUDIES IN SO-CALLED DYSPEPSIA IN BREAST-FED INFANTS

BY

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INTRODUCTION

Infants with so-called breast-fed infant dyspepsia (or, as the author terms it, colonic hyperperistalsis in neurolabile infants) have frequent, loose stools and suffer from attacks of pain. They are sensitive, whiny, and sleep badly, resulting in considerable discomfort both to the infant itself and to persons in the environment. The syndrome creates anxiety in the mother, added to which she often believes that she herself is the cause due to some fault in the quality or the quantity of her milk. Its manifestation, moreover, may at times be so severe that mothers and even physicians make the mistake of believing the syndrome to be caused by an infection. The addition of cow's milk is often given as treatment, but is accompanied by the risk of too early weaning. The care and treatment of infants with this complex of symptoms, and the education of the mothers in its causes and prognosis, is an important duty of physicians and nurses in charge of infants. Physicians are divided in their views of the causes of the syndrome and consequently of its treatment. There are therefore, from many points of view, strong grounds for investigating the nature of this type of dyspepsia.

I have personally been interested in this syndrome ever since I started work as a physician at a Children's Welfare Center some ten years ago. The addition of boiled cow's milk, which was the usual treatment, did not appear to have any effect on the complex of symptoms as such, yet was often given in increasing quantities by the mothers with weaning in consequence. I had a decided view regarding the etiology, and gave atropine and methyl atropine treatment. But not until a new spasmolytic, Skopyl (methylscopolamine nitrate), became available as a result of Nyman's (1942) investigations, and proved its suitability for infants after the good results obtained by Elgenmark (1944) in pylorostenosis, did I find a more effective and less toxic agent, with which I then began regularly to treat infants suffering from the so-called dyspepsia in breast-fed infants. At a meeting of the medical staff of the Samariten and the Sachsska Children's Hospital in January, 1949, I submitted the first results of my treatment. In order to get closer to grips with the syndrome, it was necessary to follow the events in the intestinal canal roentgenologically, and I found an interested and

stimulating associate in the then roentgenologist of the Hospital, Docent Sven-Roland Kjellberg. The first results of our cooperation were submitted at the VI International Pediatric Congress in Zürich in 1950. Since then investigations have continued and been extended, and it is this entire material, which I have also investigated clinically, that I am now presenting. The object of this paper is to submit the results of my studies, to attempt to prove the cause of this syndrome, to prove the value of the treatment, and on the basis of experience gained to discuss indications for treatment. Furthermore I wish shortly to review the more important findings and views expressed in the literature in this field, and, to the extent that I have been able to form an opinion of the physiology, to correlate the syndrome thereto.

I wish to thank all who have supported my investigations by their help and counsel. I am particularly indebted to Professor Nils Malmberg for his keen interest and valuable advice, and for the opportunity he afforded me of carrying out my investigations at the Samariten Children's Hospital; to Docent Sven-Roland Kjellberg for his ever ready assistance, interest and friendly cooperation; to Professor B. Uvnäs for his advice and help in those parts of the work which concern physiology; to Professor U. v. Euler for his assistance in and interpretation of physiological problems; to Nurse Stina Johansson at East Högalid Children's Welfare Center, to the nurses and co-workers of the Roentgenologic Department and Wards and Laboratory of the Hospital and of the Children's Welfare Centers for their interested participation in selecting and examining patients and keeping them under observation. I wish finally to thank my Hospital colleagues for their constant interest and assistance.

CHAPTER I

Earlier investigations and observations

The symptoms allied to what we in Sweden term *so-called dyspepsia in breast-fed infants* have gradually been brought together as a complex and distinguished from other gastro-intestinal affections. This complex has been presented under a variety of names that have changed with the development of our knowledge of gastro-intestinal conditions, particularly those relating to infants. Later, in the discussion (Chapter VII: J), the author comes to the conclusion that the most adequate name for the syndrome is *Colonic Hyperperistalsis in Neurolabile Infants.* This name has been used as title of the paper, but otherwise the name "so-called dyspepsia in breast-fed infants" — or abbreviated, "the dyspepsia" — has been employed.

Czerny and Moser (1894) subdivided nutritional disturbances in infants into dyspepsias and gastro-enterites, the latter due to infection, and Czerny spoke of *"Dyspepsia" in Breast-fed Infants,* using inverted commas. Epstein (1900) placed these infants who are apparently in good health in the category of *chronic dyspeptics,* pointed out diarrhea, vomitings and neurolabile symptoms, and recommended reduced feeds, strict routine and patience.

Raczynski (1902) used the term *"Die saure Darmdyspepsie der Brustkinder".* He asserted that it is a dyspepsia commonly found in breast-fed infants in the early stages, but never observed in infants fed on cow's milk. He includes among the symptoms restlessness and sleeplessness, frequent dyspeptic, acid stools, meteorism and colic attacks. He finds nothing unusual in the protein and fat contents of the milk and considers therefore that the cause is to be found in the infant itself. He made observations on women who were breast-feeding two infants at a time, one dyspeptic and one normal. Sometimes the nurse's own child was dyspeptic, sometimes the other. Thus the infants received the same nutrition, but the stools of the dyspeptic child contained a considerably greater proportion of free fatty acids, saponified fatty acids and fat. He considered that there was no other explanation for this phenomenon than a strong fermentation in the intestinal canal

and that the increased acidity in the contents of the bowels influenced the utilization of the fat. He consequently considered that intestinal disinfectants were indicated, and moreover recommended an addition of 100 Gm of cow's milk twice daily.

M a r f a n expressed great interest in this complaint and repeatedly presented his observations in papers. In 1917 he called the syndrome *"La diarrhée des enfants nourris au scin"*, a "diarrhée primitive" due to internal or external causes, but which he considered to be most often due to breast-feeding. In 1939, and earlier, he employed the term *"La diarrhée prandiale des nourrissons au scin"*. (Under the name "diarrhée prandiale", indicating the relation of the diarrhea to feeding, L i n o s s i e r (1908) had described diarrhea in connection with gallstones and gall-duct affections in adults.) Marfan gave a detailed and demarcated picture of the syndrome. He described the appearance of the feces in their different forms and pointed out that their reaction is even more acid than in normal infants due to a more lively fermentation. He found no difference in the bacterial flora compared with that of healthy breast-fed infants; at times, however, he writes, the flora in the loose stool was identical with that in the upper parts of the intestine due to a too hasty passage. He described the colic and found that these infants were often irritable, restless and fretful, and slept lightly. He attributed the gastro-intestinal symptoms to a particular congenital irritability in the intestinal tract in certain infants, provoked by human milk. He considered the possibility of a "dysergia condition" or, one may say, an "allergy condition" in certain newborn babes to human milk, and supported his assumption partly on the good results of treatment with cow's milk. He put forward two theories of the causes of these "dysergia" or "allergy" conditions: —

1. *Vagotonia.* He found this view put forward earlier principally in two works: — a. A l a r c o n (1929) based his claims on the good results he had obtained with atropine, and called the syndrome *"La dyspepsie transitoire des nourrissons"*, considering congenital syphilis a normal cause. b. C a l z a d a R o d r i g u e z (1936) submitted, under the name *"La diarrhea prandial en el lactante"*, the result of his studies and emphasized that a defect in vegetative functioning with vagal predominance is the general rule in this form of diarrhea, and that it is in most cases constitutionally sick patients who are subject to prandial diarrhea.

2. *An anaphylactic condition.* Marfan points out that no biological evidence exists for the anaphylactic origin, but reports Weill's theory that the pregnant mother resorbs products of her mammary secretion, and forms antibodies to them which are transferred to the child through the placental blood. Weill attempted by means of milk injections to desensitize infants with this affection, but with varying results.

Marfan regards his theories, however, as conjectures that remain to be proved. By way of treatment he recommends belladonna and bismuth, and possibly an "allaitement mixte".

Finkelstein (1924) spoke of a *"Physiologische Dyspepsie der Neugeborenen"*. He was careful to dissociate enteral and parenteral infections from the syndrome, and considered the cause as existing in the unsuitable quality of the breast-milk fed to the infant. He further pointed out that nutritional disturbances of this type came about due to constitutional anomalies, and found some of the infants neuropathic and in many cases their parents and siblings as well. Since he considered that some of these infants had a constitutional need of more protein and salts than other infants, he recommended an addition of cow's milk mixture or protein.

Pfaundler considered the cause of *"Der Uebergangskatarrh der Neugeborenen"* to be hypersensitivity to the irritation following on charging of the bowels. He also spoke of a *"Pseudodyspepsie bei Unterernährung"*, as did Davidsohn (1921) in *"Die initiale Diarrhöe"*.

L. F. Meyer (1924) put the endogenously caused dyspepsia in infants, i. e. disturbance *e constitutione,* in contrast to exogenously conditioned disturbances. He emphasizes that the disturbance exists during the very first days of life, and that the infant exhibits a constitutional anomaly. This is often an abnormal sensitivity in the nervous system, and it is conceivable that a vegetative neurosis is the cause of the whole complex of symptoms, particularly as the infants in many cases later exhibit signs of an abnormal irritability of the vegetative system. He gives a clear picture of the symptoms, both the gastrointestinal and those relating to the nervous disposition of the infants. His treatment consists of protein preparations.

Czerny and Keller (1928) classified the syndrome under the heading of *"Milchnährschaden der Brustkinder"*. They considered that these "injuries" might originate both from overnutrition, which the

body gets rid of through diarrhea, and from too frequent meals or change of fat content in the milk. They point out, however, that this cannot be the only cause, since a reduction in the amount of food does not reduce the dyspepsia.

Cathala (1936) wrote about *"Les diarrhées de fermentation des enfants au sein"*, the symptom picture of which is in part agreement with that of dyspepsia in breast-fed infants, but has a wider scope and may comprise disturbances in the fluid balance and, connected therewith, deep functional disturbances. He considered the cause to be an excessive sensitivity in the intestinal wall or an abnormally irritating intestinal content. Two factors are of particular importance, the gastrocolic reflex and the high content of organic acids which speeds up the intestinal peristalsis. He points to the value of breast-feeding and treats with opiates, bismuth, calc. precipite and sometimes calcium caseinate.

Brennemann (1949) describes *diarrhea* in many newborn infants with 6—10 or more stools a day, which in the breast-fed baby seems independent of the amount of food taken. It gives one the impression, he says, that the newborn baby is sometimes not quite equal at first to even the simplest food, even to specific food. He recommends that there be no change or restriction of food nor any other treatment except perhaps half a teaspoonful of castor oil. He does not associate this diarrhea with other symptoms — apart from hyperemia and even excoriations of the buttocks. In another place, however, he speaks of *colic* — the common lay expression "three months' colic" — as a symptom that has come to be looked upon as a sort of clinical entity in itself, and names three main theories which are those customarily referred to for the syndrome I am dealing with: 1. Hunger and undernourishment. 2. Overnourishment and flatulence. 3. An imbalance of the autonomic nervous system. He concludes that the rational explanation of colic is found in the fact that the baby comes into the world more "underdone" than the young of other mammals. Its intestinal tract is, moreover, during the earliest months not equal to an unusual or even normal demand, either as regards digestion or propulsion of its gaseous contents which accumulate in the intestine and are especially fostered by the highly fermentable contents of breast-milk. As a result the overdistended intestines become kinked and acutely obstructed at the bends, and the efforts to overcome this obstruction lead to colic. He does not consider atropine to be indicated, since it causes still

greater distention. Instead he gives a small soapsuds enema and, as prophylaxis, an addition of cow's milk, protein milk or the like.

J. A. Anderson (1949) writes of *colic* in artificially fed infants, recounts various causes, particularly gas, which can for example, be occasioned by hungry children sucking their fingers, indigestion, irritation of food products, food allergy or an instability in the autonomic nervous system. He refers to the expressions "neuropathic diatheses" and "hypertonic infants", and points out that the infants have a distinct personality, that they seem to respond excessively to their environment, that they are nervous, tense etc. He recommends atropine since the colic is for the most part due to the excessive accumulation of gas within the bowel and to hyperperistaltic activity.

Brackett (1950) describes *"Diarrhea in the otherwise healthy infant"*, and considers the cause to be a congenitally small capacity of the large intestine in relation to the quantity of food, or sometimes an ineffective ileocecal sphincter. He recommends small, concentrated frequent feedings.

The syndrome is not only gastro-intestinal, but also neurolabile. In referring to the above researches, I have laid most stress on the gastro-intestinal symptoms, but the majority have noticed and under various forms described the neurolabile symptoms as well, laying emphasis on them to a greater or less degree. Raczynski, even at that early stage, mentions the infants' restlessness and insomnia. Marfan finds that many infants with prandial diarrhea have a neuropathic constitution with nervosity, restlessness, insomnia etc., but in some of them he considers the symptoms due to the actual disease. He also finds a neuropathic constitution in some of the parents. Finkelstein stresses that many of the infants are neuropaths, and that migraine, nervous headaches, nervous dyspepsia, neurasthenia etc. is often found in the parents and siblings. Friedjung (1913) investigated to what degree the parents and siblings of breast-fed infants with nutritional disturbances differed from others, and found that a greater number of the former suffered from gastro-intestinal disorders. Cathala moreover considers it incorrect to classify the anomaly he has described under the heading of neuropathic diathesis. In general, attention has been given to the constitutional factors, which in Czerny-Keller's work, for instance, form an important group in gastro-intestinal affections.

Summary. The different pediatric schools have attempted in various ways to gain a proper understanding of this complex of symptoms and its different components. The view of the syndrome as both gastrointestinal and neurolabile has appeared more or less distinctly, perhaps most distinctly in L. F. Meyer and Czerny-Keller, but also in the French school headed by Marfan. Regarding the etiology and pathogenesis, the views vary considerably from that of Marfan of a congenital irritability in the intestinal tract of certain infants to breast-milk, Czerny-Keller's of the reaction to quantitatively or qualitatively unsuitable milk, Cathala's increased fermentation, Alarcon's and Calzada Rodriguez' vagotonia, Brennemann's diarrhea and colic as separate symptoms without combining them into one syndrome, to L. F. Meyer's view of a conceivable vegetative neurosis as the cause.

It is against this background that I wish to describe the investigations I have made and the results at which I have arrived.

CHAPTER II

Definition and Diagnosis

The majority of infants have 3, 4, 5 or more bowel openings a day during the early period following birth (the first week and sometimes also the two following weeks). The number of stools sometimes falls off suddenly, at others gradually. There is a sliding scale from those infants who become quickly adjusted to one or less discharges a day via those with frequent discharges during the first weeks followed by 3, 2, 1 or less a day — an adaptation phenomenon — leading to the prolonged and varyingly severe cases of so-called dyspepsia in breast-fed infants. This sliding scale makes it difficult to delimit the dyspepsia. *I decided to set the limit at an age of 3 weeks,* and the infants which at this age still have frequent, loose stools have been classified in the dyspeptic group. In some cases the remaining symptom picture must also be considered in reference to the delimitation. Such a delimitation appears to be clinically appropriate. Most babies have at that time come into a normal rhythm of their vital functions of eating, sleeping, bowel discharges etc. The intestines have during this time become adjusted to their new functions, and the intestinal flora, particularly as regards Bacterium Bifidum (in breast-fed infants), is the subsequently normal. Apart from frequent, loose, mucous and generally green stools of a prandial character, the dyspeptic infants have colic attacks, are neurolabile, as are generally also their parents and siblings, have a generally good health in spite of the symptoms continuing for weeks and sometimes for months, gain satisfactorily in weight and are without fever. In addition the dyspepsia is favorably affected by anticholinergic treatment, but not markedly by dietetic. The symptomatology is dealt with in greater detail in Chapter III.

According to the classification of nutritional disturbances adopted by Czerny and Keller, which is still mainly accepted, they are divided into three main groups:

1. *Ex alimentatione.*
2. *Ex infectione,* both enteral and parenteral.
3. *Ex constitutione.* In this group they included disturbances belong-

ing to neuropathic diathesis, e. g. rumination, pylorospasm, habitual vomiting and colic in the umbilical tract.

They also included nutritional disturbances due to *congenital anomalies,* which group also embraced hypothyreosis.

In the first group they included so-called dyspepsia in breast-fed infants, even if they observed its constitutional characteristics.

The diagnosis of so-called dyspepsia in breast-fed infants is normally not accompanied by any great difficulty. The symptomatology of dyspepsia due to infection may possibly resemble that of so-called dyspepsia in breast-fed infants. Infants with the former dyspepsia have stools possibly resembling those described above, which may be of prandial character but often occur between feedings; they may develop colic but seldom regularly and intensely as those with so-called dyspepsia in breast-fed infants; and if the disease is severe, the general health, gain in weight and temperature are affected. The infection is seldom found in the early days and is seldom of so long duration as the dyspepsia in breast-fed infants except in cases where there is evidence of other signs of infection, of the source of infection and possibly of other infected infants in the environment. An anticholinergic treatment has little or no influence on this form of dyspepsia, while a dietetic treatment is very beneficial.

CHAPTER III

Personal Investigations

1. Case material.[1]

The case material consisted of 149 infants, 111 with so-called dyspepsia in breast-fed infants and 38 normal cases, from the Samariten Children's Hospital, its Child Welfare Centers and Out-Patient Department. The dyspeptic infants have as far as possible been followed until they became free of the gastro-intestinal disorders, and many have been continuously followed later as well. The investigation includes roentgen examination and experimental treatment, and in certain cases pressure measurements in the rectum. I have attempted to renew contact with these infants later on and have made after-examinations of 117, particularly for gastro-intestinal disorders, neurolability and allergy, but only in a few cases by roentgen after-examination or by pressure measurements in the rectum. At the prior examination the age of the infants was from 1 week to 11 months, 142 being full-term and 7 premature.

a. **The 111 dyspeptic infants.** According to the degree of the dyspepsia I have classified the forms as light, medium and severe. Light cases I have classified as those lasting up to 1 month old, moderate cases up to 2 months, and severe cases those lasting beyond 2 months of age. In this may there were 27 light cases, 34 medium and 50 severe cases of the dyspepsia. This is apparent from *Table 1*, which also shows the roentgen examinations and pressure measurements that have been made. In this classification I have taken into account the duration of the dyspepsia, and to some extent its intensity but not other symptoms. In cases where the result of treatment has prevented classification on the basis of the duration of the dyspepsia, not only the intensity of the dyspepsia but also the roentgenologic findings have been taken into consideration. Difficulties have at times been encountered in the classification, and the above-mentioned sliding scale is clearly seen in the border-line cases.

[1] A specification of the cases is deposited at the Library of the Karolinska Institutet, Stockholm.

Table 1. Number of infants investigated

	No. of infants	Roentgen examination			Measurement of pressure in rectum
		Colon enema	Small intestinal passage + colon investigated	Passage through small intestine	
Normal cases	38	25	9	5	3
So-called dyspepsia in breast-fed infants	111	72	39	7	13
Light form	27	9	16	—	4
Medium form ...	34	26	7	4	4
Severe form	50	37	16	3	5
Totals	149	97	48	12	16

Feeding. Only isolated cases of so-called dyspepsia in breast-fed infants are described in which the infant has received cow's milk mixture. Of my material, 92 infants were fed solely on breast-milk, 12 on an addition of cow's milk mixture, and 7 on solely cow's milk mixture. The grouping is arranged according to the diet the infant received on coming in for examination. A mixed diet has been calculated as consisting of at least 50 Gm. cow's milk per day. Thus 82.9 per cent are purely breast-fed infants, a figure which is in close agreement with Mannheimer's material selected at random in Stockholm (1943—1947). Of these infants 83.6 per cent were still feeding at the breast at an age of 2 months without the addition of artificial food. In my investigation, the great majority of infants were under 2 months when received for examination.

Of the 111 cases of the dyspepsia, *68 are boys and 43 girls.* The predominance of boys in the severe group is remarkable.

Light dyspepsia in breast-fed infants 15 boys 12 girls
Medium „ „ „ „ 17 „ 17 „
Severe „ „ „ „ 36 „ 14 „

Marfan (1923) found that the dyspepsia existed to a greater degree in the family's first child than in the following children. In the present material, 65 per cent were first-born children, and about the same percentage of first-borns is found in my Children's Welfare Center from which a large part of the infants examined have come. The difference between Marfan's and my results is discussed in Chapter VII.

b. **The normal material consists of 38 infants,** (18 boys and 20 girls) from 1 week to 3½ months, 22 of which come from the Children's Welfare Centers. The infants have not exhibited signs of disease on examination. The other 16 infants had been taken in at the Samariten Children's Hospital. On examination their bowel functions were normal. In as far as they were hospitalized for disease, no sign of disease was demonstrable at the time of my examination. Three were received for hypogalactia or inverted nipples in the mother, three for social reasons, three for prematurity, three for observation of light icterus neonatorum of one week's duration, superficial injury by forceps (vulnus contusum) and suspected infection of the throat respectively, two for light omphalitis of which one also had dacryocystitis, and two for mild intracranial hemorrhage. Also on after-examination at the age of 14 months the two last named showed no sign of disease or injury.

Feeding. Of the normal babies 29 received solely the breast, 7 an addition of, and 2 solely, cow's milk mixture.

c. **Investigation of frequency.** To implement the above investigations, I attempted to form an opinion on the frequency of so-called dyspepsia in breast-fed infants at the Högalid Children's Welfare Center in Stockholm. The frequency investigation comprises 589 children. Of these about 30 per cent had had dyspepsia in breast-fed infants, 7.5 per cent in a mild form, 14 per cent medium and 9.2 per cent severe. In my investigation of the three oldest age groups at the Center, born 1944—1946, I made an anamnestic enquiry into "nervous" gastro-intestinal disturbances, neurolability and allergy in 139 of the children and in their parents and siblings. The enquiry is recorded in Section 5 of this Chapter.

2—4. Symptoms

The symptoms in so-called dyspepsia in breast-fed infants are the following: —

Gastro-intestinal symptoms (see Sect. 2 A and B). In this group of symptoms should perhaps be included the *intertriginous lesions* on the buttocks of many of the infants (see Sect. 3).

Neurolabile symptoms (see Sect. 4).

Apart from these symptoms should be emphasized the infants'

months with abdominal pains, called "three months' colic", but in the few cases discovered so-called dyspepsia in breast-fed infants was present at the time or had existed earlier. It appears likely that the majority of cases of "three months' colic" have suffered from the dyspepsia in breast-fed infants and that the frequent, loose stools have disappeared.

Abdominal pains in cases of the dyspepsia may be so intense and sudden, at times being accompanied by vomiting, that there is suspicion of invagination. The number of cases in which the mother has reported such attacks — medical advice having generally been sought — is 18. One of these cases had an invagination.

c. **Gases.** In medical literature is stated that flatulence and meteorism often occur in cases of the dyspepsia. Many of the mothers have spontaneously complained that their babies had gases, particularly during feeding times, and that there were rumbles in the stomach which they interpreted as gases. In about 80 per cent of the dyspeptic cases a more or less severe flatulence has been observed by mothers, as against 60 per cent in the normal infants. Of the cases of severe flatulence there was only 1 normal infant out of 26 with the dyspepsia. A comparison of the amount of gas visible in the first roentgen examination, however, reveals no distinct difference in relation to normal infants. No pronounced meteorism or appreciable divergence in relation to normal infants in this respect was demonstrable in the investigation. The dyspeptic infants on the other hand often have *an increased tonus* in the abdominal muscles.

d. **Vomiting.** Many mothers complain that their babies suffer from vomiting, and the investigation showed that moderate or severe vomiting occurred in 24 out of 87 reported cases. These symptoms have not been observed in the normal infants, while regurgitations occur about equally in the normal and dyspeptic infants. After-examination revealed that 5 of the dyspeptic infants had later visited a doctor for rumination, but this symptom has not been observed in any of the normal infants.

e. **The duration of gastro-intestinal symptoms.** The classification of the dyspepsia into light, medium and severe forms is principally based on the duration of the dyspepsia. The light and medium cases are in

this respect normalized at an age of 1 and 2 months respectively. Many of the severe cases have been treated, and a tabulation of the duration of dyspepsia would be misleading. All variations of duration are found in the severe case material, from infants with loose stools for rather more than 2 months to others extending over 6 months. The majority, however, were normalized at an age of 3—4 months. The duration of the pain attacks varies similarly to that of the loose stools, but the former often extend over a longer period.

f. **Late symptoms from the gastro-intestinal tract.** The infants examined are still too young for a knowledge to be gained of how their gastro-intestinal tract will function later on. I have, however, observed and pointed out the marked sensitivity of the digestive canal of the infants, and in an investigation at the Högalid Children's Welfare Center — see Section 5 of this Chapter — I have found a tendency in cases of so-called dyspepsia in breast-fed infants to symptoms similar to those observed in *spasmus coli.*

2 B. Symptoms from the gastro-intestinal tract in the parents and siblings

So-called dyspepsia in breast-fed infants — as I am able to establish later in Chapter VII — is probably due mainly to "nervous" factors. As it was conceivable that the parents and siblings would also have gastro-intestinal disturbances of a psychosomatic nature, I carried out an investigation and was able to establish that they suffer from certain nervous disturbances proceeding from the digestive canal to a higher degree than the families of normal infants (see *Table 3*).

Whether the parents have suffered from *so-called dyspepsia in breast-fed infants* is difficult to determine. Many of the grandmothers have after so many years forgotten the state of their children's health, and many are no longer alive. But many others have spontaneously declared: "Just think how difficult you were as a child!" At least 13 of the parents of the infants examined had presumably had the dyspepsia as against none of the parents of the normal children. Of the siblings of the dyspeptic infants, 19 out of 47 had had the dyspepsia, as against 2 out of 9 siblings of the normal infants.

In the anamnesis have also been included mild cases of "nervous" gastro-intestinal disturbances, patients who had had pains of the

Table 3. "Nervous" gastro-intestinal disturbances in parents and siblings

	Infants with so-called dyspepsia in breast-fed infants			Normal infants		
	Mother	Father	Siblings	Mother	Father	Siblings
No. of cases investigated	111	111	47	38	38	9
No information	9	8	—	1	1	—
So-called dyspepsia	7	6	19	—	—	2
Pains of spasmus coli type ... (as a child)	35	2	13	2	—	1
Irritable colon,						
mild form	17	4	1	3	2	—
medium form	32	13		4	—	—
severe form	19	5		1	—	
Gastric catarrh or gastric ulcer	5	9		—	3	
Constipation	10	6		2	1	
"Nervous" gastro-intestinal troubles in other relatives ..	12	10		—	—	

spasmus coli type during infancy, or who on particular occasions later in the course of travels, visits to doctor or dentist and so on, had suffered from acute discomfort in the pit of the stomach, or from pains or diarrhea; or who, in short, had in varying degrees suffered from *irritable colon* or nervous diarrhea. Gastro-intestinal disturbances in the mothers, in particular, have been observed from the commencement of the investigations, since it was a normal occurrence that they arrived late for roentgen examination. On enquiry they often replied that they had had to visit the toilet just as they were about to leave home. But the nervous gastro-intestinal sensitivity of the fathers is also often recorded. In two cases of babies which later proved to have the dyspepsia extremely severely, the fathers had been so affected gastro-intestinally at the time of the child's birth that they had to be absent from work with acute stomach pains or diarrhea and vomiting.

Constipation should perhaps also be mentioned in this connection. I did not observe constipation from the commencement, and the tabulated figures should therefore be taken as minimum values. It appears, however, to be a normal phenomenon in the parents of these infants.

Pains in the region of the gall-bladder are also mentioned by several in the anamnesis. In cases where *gastric ulcers* or *gastric catarrh* existed, the Table only includes patients who had consulted a doctor.

Many of the mothers have spontaneously reported that disorders of the gastro-intestinal canal, similar to those described above, had occurred to a high degree in one of their parents or siblings; but many have so little knowledge of their relatives that the results tabulated are to be regarded as minimum figures.

"Nervous" gastro-intestinal troubles, or a disposition to such, may be to a greater or less degree *hereditary*. Of the infants examined, 17 of the cases of dyspepsia in breast-fed infants were sole members of the family to have such disturbances. In 49 of the remaining 85, only one of the parents have now or previously exhibited gastro-intestinal disturbances of a nervous character, while in 23 both parents, and in 13 one parent and one or more siblings, had exhibited such disturbances (see *Table 4*). In *Table 4* is also recorded a similar investigation of the normal material. In 8 cases one of the parents or one sibling had had "nervous" gastro-intestinal disturbances, while only isolated cases exist of these disturbances in more than one member of the family. The same observation was made as regards the infants examined at Högalid Children's Welfare Center (see Section 5 of this Chapter).

Table 4. Occurrence of "nervous" gastro-intestinal disturbances (so-called dyspepsia in breast-fed infants, spasmus coli, irritable colon and gastric catarrh or gastric ulcer) in families of dyspeptic and normal infants

	Infants with so-called dyspepsia in breast-fed infants	Normal infants
None of the parents or siblings	17	26
One parent or one sibling	49	8
Both parents (one child in family)	16	1
Both parents (more than one child in family)	2	—
One parent + sibling(s)	13	1
Both parents + sibling(s)	5	1
No information	9	1
Totals	111	38

3 A. Cutaneous lesions and allergic manifestations in the infants

Among the causes of dyspepsia in breast-fed infants, some researchers consider allergy as a possible factor. Allergic skin manifestations are usual in infants, while manifestations from the respiratory passages are less common. Symptoms from the gastro-intestinal tract are rare. In nursing infants, sensitivity to human milk often results from the transmission of allergens from the mother's diet to her milk. It is not seldom found, for instance, if the mother eats strawberries, that the child develops allergic manifestations in the skin, mostly on the face, while disturbances of the gastro-intestinal canal are rarely observed.

Among the 149 infants examined, in only one case had the mother observed that the child's gastro-intestinal symptoms might possibly be connected with her own diet. In this exceptional case the mother thought that her child suffered more severely from gripes when she had eaten

Table 5. Intertriginous lesions, allergic or suspected allergic manifestations in the infants investigated

(At the time of investigation or later)

	Infants with so-called dyspepsia in breast-fed infants	Normal infants
No. of cases investigated	111	38
Infantile eczema,		
mild	13	6
medium	3	4
severe	—	—
Intertriginous lesions,		
mild	40	6
medium	7	—
severe	1	—
Urticaria,		
mild	4	3
medium or severe	—	—
Chronic eczema,		
mild	1	2
medium or severe	1	—
Allergy of respiratory passage,		
asthmatic bronchitis	1	—
Allergy of gastro-intestinal canal	—	—

peas or red beans. After weaning, the child exhibited no gastro-intestinal symptoms other than diarrhea on one occasion due to infection. On one further occasion it developed small spots on the face after receiving a large amount of orange juice. It should be pointed out that, as a result of their children's ailments, the mothers are on the look out for changes and many have of their own accord made dietary experiments with no subsequent change in the symptoms of the child. Even on after-examination of these children I have not found a single case with allergic manifestations from the gastro-intestinal canal. No case of cow's milk idiosyncrasy was met with.

Intertriginous lesions on the buttocks due to the frequent, loose, acid stools are a common phenomenon in these infants. These lesions do not appear to be of an allergic nature and they heal rapidly on appropriate treatment. The infants covered by the investigation do not exhibit a greater degree of allergic manifestations than the normal children, as can be seen from *Table 5*. This Table also includes cases which on some single occasion had a skin reaction, even if it were only a mild reaction on the cheeks, and in which the allergic factor could not be established.

Table 6. Allergic or suspected allergic manifestations in parents and siblings of the infants

(At the time of investigation or earlier)

	Parents and siblings of					
	Infants with so-called dyspepsia in breast-fed infants			Normal infants		
	Mother	Father	Siblings	Mother	Father	Siblings
No. of cases investigated	111	111	47	38	38	9
No information	6	7	—	1	1	—
Allergic or suspected allergic manifestation in						
Skin	9	5	6	9	1	3
Respiratory passage	2	2	2*	—	—	1**
Gastro-intestinal canal	—	—	—	—	—	1***

* Asthmatic bronchitis in 2 siblings.
** Half-brother asthmatic.
*** Diarrhea from chocolate and pears.

Only one infant with suspected allergy of the respiratory passages has been met with in after-examinations, the infant having had an asthmatic bronchitis and the mother asthma as a child. The infant had earlier had a rather troublesome form of the dyspepsia.

3 B. Allergic manifestations in parents and siblings

Medical literature makes it clear that heredity is one of the most important predisposing factors in the pathogenesis of human allergy. Spain and Cooke have shown that infants with a bilateral antecedent history develop allergy in about 75 per cent of the cases; those with a unilateral history in one-half of the cases. Where there was a bilateral history, symptoms appeared in the first 5 years of life among 36 per cent, while those with only one allergic parent developed allergy later.

The results of the examinations of parents and siblings are represented in *Table 6*. As in the examination of the infants, the material includes allergic or suspected allergic manifestations. With the exception of one case, the skin lesions were of a mild nature. Only one case of gastro-intestinal allergy, in a sibling of a normal infant, was observed, diarrhea developing regularly after chocolate and pears.

The incidence of allergy or suspected allergy in the family (intertrigo excluded) is represented below: —

	Cases of the dyspepsia	Normal infants
Number of infants in investigation	111	38
No information	7	1
Allergy solely in infant	13	6
„ „ „ one parent	7	3
„ „ „ sibling(s)	2	—
„ „ „ parents	—	1
„ in infant + one parent	7	3
„ „ „ + sibling(s)	2	3
„ „ „ + parents	—	1
„ „ „ + one parent + sibling(s)	2	1

The above figures show that none of the dyspeptic infants had a bilateral antecedent allergic history, while 7 had a unilateral history, as

against 1 and 3 respectively of the normal infants. There is thus no increase in allergic heredity in infants with the dyspepsia in relation to the normal infants.

4 A. Neurolability in the infants

In the great majority of cases of so-called dyspepsia in breast-fed infants a neurolabile disposition is found, which only exists in rare cases among the normal material. The most striking feature of the neurolabile symptoms is the excessive sensitivity to sound. The infants start at a little click of the roentgen apparatus, and the sound of a voice or scraping of a chair produce long, uncontrollable screaming. They are awakened and begin to cry inconsolably at the least sound such as stealthy footsteps or a creaking door. Many mothers have observed also that their babies are sensitive to light, that they wake and scream if the light is turned on and that they cannot go to sleep if it is light in the room. Their sleep is shorter and less sound than that of normal infants; they sleep in short bouts by day and their screams at night are often disturbing to the household and neighbors. They are in general impatient and irritable. They have difficulty in relaxing, are wakened on the least occasion, become fretful and impatient and take a long time to calm down again. They twist and turn, kick and display motor restlessness in general. Isolated cases have found it difficult to learn to take the breast. Some have become taut and have turned away, and have sucked poorly when settled into position, and a number have shown lack of endurance in sucking the breast. Sometimes the mothers have complained of their babies sweating, often localised to the hands, neck, head or feet.

A number of these symptoms are more marked at times of gastro-intestinal disorders; crying, restlessness and poor sleep may be partly attributed to such disorders. But even when the infants' intestinal functions have become normal, there is in the main a continuance of the symptoms. As they grow older moreover, new symptoms of their sensitivity appear, an exceptional promptness in reaction and intensity of reaction, while other symptoms fall away. When the time of weaning arrives, they often take longer to learn to feed by spoon, are impatient and contrary. Particularly significant is their fear of any novelty, of strangers etc. They often cry continuously during visits to the Welfare

Center, and this reaction appears to manifest itself earlier the more severe the neurolability. As soon as they have grown to a certain stage of development, they fight madly against any attempt at examination and when laid to rest on the mother's knee seek refuge in thumb-sucking. The older ones often develop calluses and other sores as a result of forceful thumb-sucking, and later sometimes exhibit signs of nail-biting. Their sensitivity also extends to the vegetative nervous system, and their gastro-intestinal disorders, provoked later by mental strain, are dealt with in Section 5 b of this Chapter.

At the very first examination of these infants, most of which were aged 2—6 weeks, their neurolabile disposition was demonstrable, and the mothers have very often noticed their sensitivity. The symptoms are present in the first week, but often increase during the following weeks as the infants develop and acquire the capacity to react more intensely, possibly also due to the advance of gastro-intestinal disturbances, but undoubtedly to environment as well. The mothers of the dyspeptic infants are often tense, nervous and anxious about their offspring, particularly on account of their at times quite alarming gastro-intestinal symptoms. Several mothers have pointed out the influence of environment since they found that the infants became calmer, slept better and cried less when they were put in a separate room. This was all the more marked in the infants taken into the Hospital. The crying and restlessness often diminished and the infants learnt to sleep at night, sometimes within a few days and sometimes after a longer period. But the sensitivity to sound and light remained, the infants were still irritable and sensitive, and their crying and sleeplessness often returned when they had been at home a short time. I shall be returning to the question of environment in Chapter VI on treatment.

The after-examination comprises 87 cases having suffered from so-called dyspepsia in breast-fed infants and 30 infants in the normal group. The majority were aged 1 to 2 years, but some younger ones were included ranging down to 3 months. The neurolability was found to be still present, but with features typical of their respective ages. Their neurolability was, however, in many cases less severe than it had been in the period of gastro-intestinal disorders, and hardly a single child still exhibited the almost intolerably enhanced sensitivity, restlessness and crying fits which had often accompanied the really severe intestinal disorders. The circumstance that an improvement had in many

cases occurred in the infants' neurolability indicates that the gastro-intestinal disturbances in these infants often accentuate the neurolabile symptoms.

I have attempted to form an idea of the infants' neurolability on the basis of my own and the nurses' observations and of the mothers' statements. The different forms have been classified as mild, medium and severe. Account has been taken of whether the infant exhibited one or more of the pertinent symptoms, and of the degree of severity of the respective symptoms. *One* classification group was compiled on the basis of the mothers' observations and statements, and *one* on the basis of

Table 7. Neurolability in the infants

	Infants having or having had so-called dyspepsia in breast-fed infants			Normal infants
	Light	Medium	Severe	
A. At the time of gastro-intestinal disturbances				
1. *Based on statement of the mother*				
No or mild neurolability	5	4	3	31
Medium „	12	18	11	6
Severe „	9	12	35	—
No or unreliable statement	1	—	1	1
	27	34	50	38
2. *Based on my own and nurse's opinion*				
No or light neurolability	5	3	3	31
Medium „	13	19	8	6
Severe „	8	12	38	—
No or unreliable statement	1	—	1	1
	27	34	50	38
B. At after-examination				
Based on statement of the mother + my own and nurse's opinion				
No or light neurolability	9	3	1	21
Medium „	9	16	14	8
Severe „	5	8	22	1
No or unreliable statement, i. e. infants too young or too recently examined to be after-examined	4	7	13	8
	27	34	50	38

the observations and appraisal of myself and the nurses. There were only small and insignificant deviations between the two groups. The results are recorded in *Table 7*. About 90 per cent of the dyspeptic infants exhibited medium or severe neurolability during the period of their gastro-intestinal disorders. There is a significant parallelism between the degree of neurolability and of gastro-intestinal disturbance. The marked occurrence of neurolability in these infants must signify that the predisposition to so-called dyspepsia in breast-fed infants exists principally in this group of newborn. By way of comparison it is interesting to note that none of the normal infants exhibited a severe neurolability on the first examination, and only 6 out of 37 (16.2 per cent) a medium neurolability. But on after-examination the degree of neurolability had decreased in the dyspeptic infants, while the contrary is the case as regards the normal infants, which corroborates my assertion above that gastro-intestinal disorders may accentuate the neurolabile symptoms. The role played by environment is difficult to

Table 8. Neurolability in parents and siblings of the infants

	Parents and siblings of			
	Infants with so-called dyspepsia in breast-fed infants			Normal infants
	Light	Medium	Severe	
Mother				
No or mild neurolability	8	8	6	26
Medium „	11	14	24	8
Severe „	7	10	19	3
No information	1	2	1	1
	27	34	50	38
Father				
No or mild neurolability	17	27	36	34
Medium „	6	3	9	3
Severe „	3	2	1	—
No information	1	2	4	1
	27	34	50	38
Siblings				
No or mild neurolability	6	11	5	6
Medium „	1	3	8	2
Severe „	3	2	5	1
No information	1	1	1	—
	11	17	19	9

assess, but it is a remarkable fact that the infants often exhibit this pronounced neurolability while still in the Maternity Ward and on their arrival in the home, a circumstance which throws the hereditary element into strong relief.

4 B. Neurolability in parents and siblings

I also attempted to form a judgement of the neurolability of parents and siblings, both by talks with the mothers and by the nurses' observations, the groups being classified as before (cf. *Table 8*). The majority of mothers have a neurolabile disposition (about 80 per cent), in which they differ from the mothers of the normal infants (about 31 per cent). Only 23 per cent of the fathers are recorded as neurolabile as against 8 per cent of fathers of the normal infants, but the figures have been compiled practically entirely from mothers' statements. The siblings too, compared with siblings of the normal infants, were more often neurolabile.

The number of neurolabile parents and siblings is remarkably high and indicates a hereditary tendency to a neurolabile constitution in the dyspeptic infants.

5. Supplementary investigations at the East Högalid Children's Welfare Center in Stockholm

To implement the aforegoing investigations I attempted to form an idea of the frequency of so-called dyspepsia in breast-fed infants at the East Högalid Children's Welfare Center in Stockholm. I can find no reason why the attendances at the Center should not be representative of the population of Stockholm. Enquiries as regards children born in the years 1944—1946 (139 children) were answered by the mothers covering the gastro-intestinal anamnesis of the child, both when new-born and later, allergic manifestations and neurolability; and as regards the parents and siblings covering nervous gastro-intestinal troubles, allergy and neurolability. As regards children born in the years 1947—1951 (450 children), the only data recorded here are whether they had had the dyspepsia and whether allergic manifestations were present. The data were obtained from the journal entries of the infants. Data on all infants are implemented by information from the Nurse in charge of the Welfare Center. The investigation comprises 589 children. All the results are recorded in *Table 9*.

Table 9. Investigations at the East Högalid Children's Welfare Center in Stockholm

Frequency of the so-called dyspepsia in breast-fed infants, "nervous" gastro-intestinal disorders, allergic or suspected allergic manifestations and neurolability in the children, parents and siblings

	Number of children examined	Infants with so-called dyspepsia in breast-fed infants	Infants without the dyspepsia
A. Children born 1944—1951			
a. *Frequency of so-called dyspepsia* ..	589	181	408
Mild form		44	
Medium form		83	
Severe form		54	
b. *Frequency of allergic or suspected allergic manifestations*	589		
Eczema, mild form		22	67
medium-severe		7	28
Urticaria		7	11
Respiratory passage		1	3
Gastro-intestinal canal		—	—
B. Children born 1944—1946	139	35	104
a. *Noticeable tendency to diarrhea later*		7	2
b. *Pains of spasmus coli type*			
Mild form		1	5
Medium form		8	12
Severe form		5	—
c. *Neurolability*			
None or mild form		5	68
Medium form		20	27
Severe form		10	9

Table 9 (continued)

	Parents or siblings of	
	Infants with so-called dyspepsia	Infants without the dyspepsia
C. Parents and siblings of 139 children born 1944—1946, of whom 35 have had so-called dyspepsia in breast-fed infants, and 104 have not		
a. *"Nervous" gastro-intestinal disorders*		
Mother:		
Mild form	2	27
Medium form	14	
Severe form	2	
Father:		
Mild form	1	18
Medium form	7	
Severe form	4	
Number of Siblings:	24	62
Mild form	—	12
Medium form	3	
Severe form	3	
b. *Allergic or suspected allergic manifestations*		
Parents	8	15
Siblings	4	4
c. *Neurolability*		
Mother:		
Mild form	8	18
Medium form	12	16
Severe form	8	9
Father:		
Mild form	8	7
Medium form	4	10
Severe form	5	5
Number of Siblings:	24	62
Mild form	1	19
Medium form	5	
Severe form	2	

a. **Frequency.** It was pointed out earlier that a sliding scale pertains from normal condition to an increasingly pronounced so-called dyspepsia in breast-fed infants. If the duration and to some extent the intensity of frequent discharges are taken into account, and the demarcation between normal and dyspeptic infants is put at an age of 3 weeks, i. e. normalization being considered as occurring then or earlier, about 30 per cent of the infants have had the dyspepsia. If the demarcation is instead put at an age of 1 month — in the aforegoing stated as the demarcation between light and medium cases — the percentage is 23, and if the severe cases alone are counted, i. e. with a longer duration than 2 months, the figure is 9.2 per cent (see *Table 9 A*).

b. **Symptoms from the gastro-intestinal tract.** The same observations were made here as in regard to the case material. A marked predisposition to diarrhea after normalization appears, and of the infants born 1944—1946 this predisposition was found in 20 per cent of the formerly dyspeptic infants as against 2 per cent of the normal infants.

More than one-half of the infants had pains during or after feeding while suffering from the dyspepsia. In about half of the cases the attacks persisted after the dyspepsia had receded. Only 2 per cent of the remaining children exhibited similar attacks, but had loose discharges on feeding, which ceased before the 3-week limit was reached. This investigation also supports my supposition, referred to earlier, that the majority of infants with "three months' colic" had had the dyspepsia and that "three months' colic" is a remnant of the dyspepsia.

The mothers have not seldom complained during the course of the years that their children have had gripes of varying severity, sometimes followed by temporary loss of appetite and sometimes combined with tendencies to vomiting or diarrhea, when the infants were subjected to mental stress. These symptoms are presumably often due to a spasmus coli and probably correspond to irritable colon in adults. Troubles of the kind appear among the infants investigated, particularly in the formerly dyspeptic infants where they are also more intense, which is corroborated in *Table 9 B*.

Symptoms from the gastro-intestinal tract in the parents and siblings. It has been established earlier in the case material that the parents and siblings of the dyspeptic infants have exhibited "nervous" gastro-

intestinal disturbances to a higher degree than those of the normal group. The circumstances are similar in the results from the Children's Welfare Center, where an inclusion has been made in the same way of mild cases of "nervous" gastro-intestinal disturbances, i. e. so-called dyspepsia in breast-fed infants, pains of the spasmus coli type in infancy, irritable colon, nervous diarrhea etc. Over 50 per cent of mothers of the dyspeptic babies have or have had "nervous" gastro-intestinal troubles as against 26 per cent of the mothers of the normal group, the figures of fathers being 34 and 17 per cent respectively (see *Table 9 C*). If the infants which have had ailments similar to spasmus coli are not included in the normal group, thus freeing the material of other cases too where hereditary "nervous" gastro-intestinal disturbances are conceivable, the frequency of "nervous" gastro-intestinal disturbances is reduced to 15 per cent for mothers and 10 per cent for fathers.

Heritability of "nervous" gastro-intestinal disturbances. Of the 139 infants investigated, born in the years 1944—1946, the dyspepsia was found in varying degrees in 35 cases, of which 21 were boys. Of these 35 infants, there were 24 (70 per cent) with one member of the family who had suffered from "nervous" gastro-intestinal ailments, including 8 with more than one member. There were 81 infants who had had no intestinal troubles. Of the latter there were 26 (32.1 per cent) with one member of the family who had or had had "nervous" gastro-intestinal ailments, and 4 with more than one member. As was found earlier in the case material, there was a marked concentration at the Children's Welfare Center of "nervous" gastro-intestinal disturbances in the parents and siblings of children who had had the dyspepsia. This circumstance is the more remarkable considering that figures are recorded from a whole Welfare Center and not merely from selected material.

c. **Allergic and suspected allergic manifestations.** Mild skin reactions were included in this investigation as well, even if the allergic factor could not be established. A large number of infants will therefore be counted as allergic or suspected allergic, about 20 per cent of the dyspeptic infants and about 25 per cent of others. I thus do not find here either any evidence that the dyspeptic infants have allergic or suspected allergic manifestations to a higher degree than the normal infants.

Parents and siblings of the dyspeptic infants on the other hand

exhibit a proportionately greater number of cases of allergic or suspected allergic manifestations in the skin and in the respiratory passages than parents and siblings of the normal infants (see *Table 9 C*).

d. **Neurolability.** At the Child Welfare Center too attempts have been made, similar to those described earlier, to obtain an opinion on the infants' neurolability. A neurolability of varying severity appears in the majority of the infants (born 1944—1946) which had had the dyspepsia. The number of neurolabile cases increases with growing age. This increase may be due to the greater difficulty in assessing neurolability in the infant stage and to the fact that the mother, now that at least four to five years have passed, only remembers the most distinct symptoms. An important factor, however, may be the influence of environment. The normal infants also exhibit similar tendencies. At the time of the investigation it proved that about 85 per cent of the formerly dyspeptic infants were neurolabile as against about 35 per cent of the normal infants (see *Table 9 B*).

In this material, in which the future gastro-intestinal history of the infants can be reviewed to some extent, it is interesting to examine the connection between neurolability and "nervous" gastro-intestinal disturbances. It is shown that, where some member of the family has or has had "nervous" gastro-intestinal disturbances, 80 per cent of the infants are neurolabile as against 7 per cent of the other children. The results suggest an intimate connection between neurolability and a tendency to both the dyspepsia and other "nervous" gastro-intestinal disturbances.

Parents and siblings of infants which have had the dyspepsia often exhibit a neurolabile disposition, as has also been described above. Thus a moderate or severe neurolability is found in about 55 per cent of the mothers and about 25 per cent of the fathers as against 25 per cent of the mothers and 15 per cent of the fathers of the normal infants. In forming an opinion I have mainly followed the information provided by the mother.

CHAPTER IV

Roentgenologic Studies

In order to penetrate more deeply into the patho-physiology of the gastro-intestinal tract in cases of so-called dyspepsia in breast-fed infants, roentgenologic examination is necessary, and I have had the benefit of carrying out studies in cooperation with Docent Sven-Roland Kjellberg. The gastro-intestinal activity of 105 of the dyspeptic infants and of 35 normal infants has been examined (see *Table 1*, p. 16). A more detailed account of the roentgen examinations is published in another journal.

Procedure. Two different methods have been employed in the roentgen examinations.

a. For the study of the stomach, duodenum and small intestine, the infants received a barium contrast medium *by mouth* (5 teaspoons). The medium used, Pharmabaryt (AB Pharmacia, Uppsala), has in most cases been diluted in the normal manner with water, but on some occasions when the infant was very small, breast-milk was used for breast-fed infants instead of water, and cow's milk mixture for infants on that diet. The temperature was the normal for infants' food, about 35° C. The gastro-intestinal canal was subsequently studied fluoroscopically during feeding. Roentgen pictures were taken on suitable occasions. The shortest possible screening time was used in order to protect the infant from the X-rays, and did not extend beyond max. 5 minutes. For the same reason the number of pictures was kept as low as possible. Sixty infants were examined by this method.

b. In roentgen study of the colon, a barium contrast medium (Pharmabaryt) was given either *by mouth* or as an *enema*. For this purpose a special catheter (Ameran acmi 20, inflate 200 cc), was used, which prevents the contrast enema from running out when the pressure in the intestine increases. The temperature of the enema was about 37° C. When the colon had been adequately filled with contrast medium, the catheter was removed and for the following 5 minutes the infant was left to discharge the enema. If, at the end of a 5 minute

period, the infant continued to discharge the enema, or if it was observed by fluoroscopy that movement continued in the colon, the period was extended. An examination of 97 infants was made by this method.

A. Investigation of ventricle, duodenum, small intestine and colon

1. Stomach

In order to be able to study the appearance of the ventricle the infant was given 5 teaspoons of barium contrast medium. During screening the infant was breast-fed or fed by other methods. No difference between dyspeptic and normal infants was demonstrable. The amount of gas was practically the same in both categories.

2. Duodenum and small intestine

As soon as the contrast medium had filled the duodenum, the infant was given the breast or other type of food. The appearance of the peristalsis and the passage of the contrast medium were studied both before, during and after feeding. A continuous check was subsequently made at one to two hour intervals, and food was given at every examination.

About 15 seconds after the commencement of feeding a vigorous movement started in the duodenum which quickly forced the contrast medium forward. This movement spread quickly to other parts of the small intestine (see *Plates 1 a—c*). When the bowel movement had reached the lowest part of the ileum about 1½ minutes after the commencement of feeding, this part of the intestine began to contract, and at times parts of the contrast medium were seen to be squeezed through the ileocecal sphincter, the latter appearing as a 2—5 mm wide passage between the two parts of the intestine. Towards the end of the feed, and particularly afterwards, the bowel movements decreased. No difference was observed in the pictures of the dyspeptic and the normal infants — apart from the fact that the ileocecal sphincter was not observed in any of the normal infants, but a replenishment of contrast medium in the cecum during the meal — nor between different kinds or methods of feeding.

Transit time. The time from the start of the contrast medium being applied until it was first observed in the colon was registered. The num-

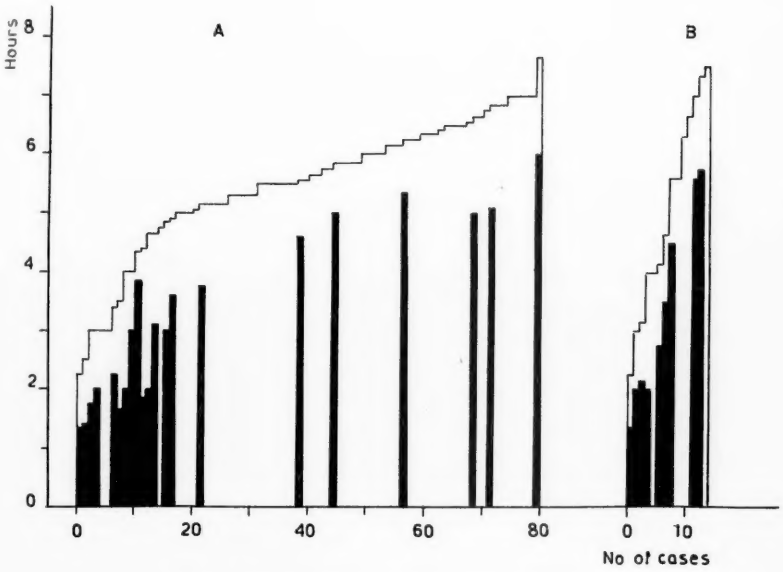


Fig. 1. Time of transit through small intestine.
A. In infants with so-called dyspepsia in breast-fed infants.
B. In normal infants.
The upper indented lines indicate the time from the contrast medium being administered until it is first observed in the colon. The black columns indicate the moment of the last observation, when the contrast medium was in the small intestine only.

ber of observations made of the dyspeptic infants was 80 for altogether 45 infants, while for normal infants the number of observations and infants was 14. No difference in the transit time as between the two groups was demonstrable. *The transit time varies in round figures between two and seven hours* in both groups. The results are given in *Fig. 1*, which also shows the time of the last observation before the contrast medium reached the colon, in cases where such observations were made. The result is in agreement with H e n d e r s o n's observations (1942) of newborn babies, in which the transit time varied between two and six hours, and with L ö n n e r b l a d's studies (1951) of healthy one-year-olds with the transit time varying between 1½ and 6 hours.

3. Colon

Roentgen examination of the colon was, as stated above, done by two methods. When infants with the dyspepsia received a barium enema, the infusion was often accompanied by considerable difficulty. In pronounced cases, the colon contracted so violently that the pressure in the contrast enema had to be increased from the normal 50 cm. water to 80 cm. and above. When the catheter was removed, the contrast enema often left the anus in a regular cascade. In severe cases the colon contracted very violently and forced out almost all the contrast medium within a short time, leaving the colon appearing like a thin string (see *Plates 3* and *4*). In less severe cases on the other hand a varying amount of contrast enema remained in the colon (*Plate 5*). When a normal infant was examined by the same method, the colon infusion proceeded with perfect ease. The colon dilated to a considerable width, and after defecation there remained fairly large amounts of contrast medium in the comparatively distended colon (*Plate 2 a*). If the barium was given by mouth, part of the contrast medium had penetrated to the colon by the time the examination commenced. At times, however, the examination had already started when the forward portion of the contrast medium had only reached the lower ileum.

Once the possibility existed of following the appearance and movements of the colon, these were studied during the infant's feeding. Before feeding, and during the early part of the meal, no — or only insignificant — motility was found in the colon. To the extent that contrast medium is present in the small intestine, the movement commences there earlier than in the colon. About 1½ minutes after the commencement of feeding, more or less violent contractions are observed in the cecum or at times in other parts of the colon of the dyspeptic infants, which force the contents forward — in severe cases very rapidly, and more slowly in others. The movement generally spreads, and when it reaches the sigmoid colon a convulsive contraction is often observed which sometimes embraces only a part of the sigmoid colon but at other times larger or smaller parts of the descending colon also. The convulsive contraction which closes part of the colon forces out all the contrast medium, and no further passage of contrast medium can take place. It is often noticed that, simultaneously with the onset of the contraction, the infant's leg goes up like a signal arm in the field of vision, indicating that its limbs contract with pain, it ceases feeding,

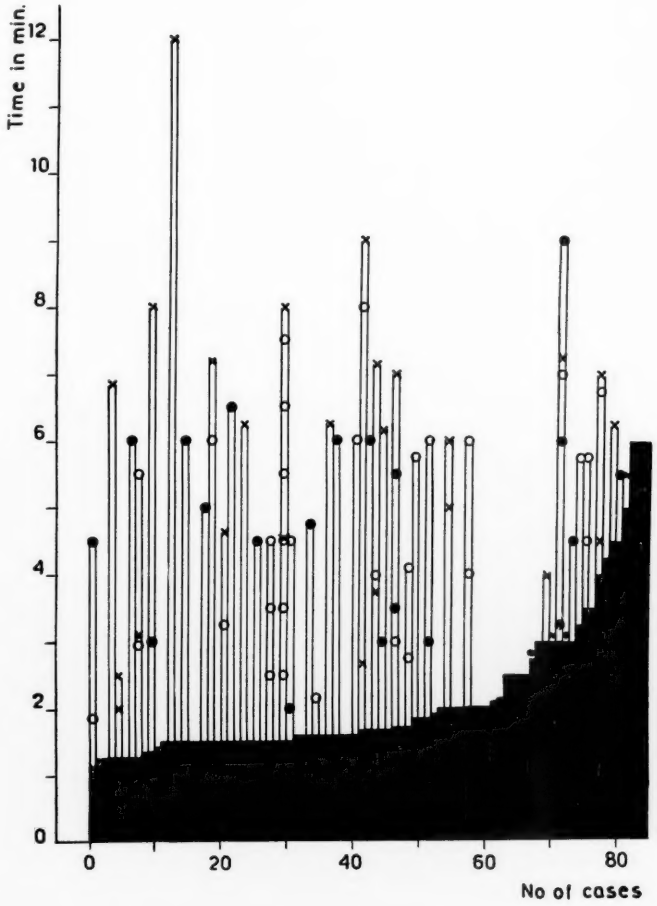


Fig. 2. Time of commencement of propulsive motility of the colon, new peristaltic waves, onset of pain and defecation. Time counted from commencement of feeding.

Each column represents one infant.

The lower dark area indicates the time of commencement of propulsive motility of the colon.

o = time of commencement of new propulsive motility of the colon.

• = time of onset of pain attacks.

x = time of defecation.

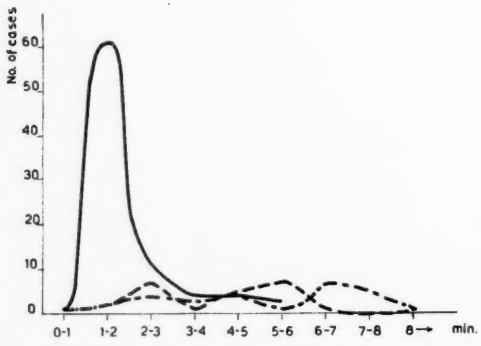


Fig. 3. Frequency curve from Table 2.
——— = time of commencement of propulsive motility of the colon.
— — — = time of onset of pain attacks.
— · — · = time of defecation.

whimpers or yells and squirms. In the same moment that the convulsive contraction passes away, the infant becomes calm and resumes the feed. On some occasions, however, the condition of contraction remains, and the infants are irritable and peevish during the remainder of the examination. In some cases the attacks of pain commence regularly 2—3 minutes after feeding has begun, but at times a little later, e. g. 5—6 minutes. The intense colonic peristalsis is generally concluded by one or more explosive discharges, which often commence 6—7 minutes after the beginning of the meal. It is sometimes noticeable that one or more new propulsions occur at intervals of one or a few minutes, whereupon the same scene is repeated as described above. This is, however, not always the rule, but sometimes a condition of contraction remains throughout the colon after the first propulsive phase. On rare occasions a contraction has commenced throughout the colon or in several parts of the colon simultaneously, on occasions also accompanied by propulsion, pain and defecation, practically all at the same time. *Fig. 2* shows the time of commencement of the peristalsis, of the onset of pain and defecation, and a still clearer picture is given in *Fig. 3*.

On rare occasions it was found that a baby with the dyspepsia, which had been very whiny and contrary during the examination, had not reacted with propulsive colonic motility in spite of being fed.

Varying amounts of gas have been observed in the colon without demonstrable difference between normal and dyspeptic infants.

In many cases an infant that had undergone earlier examination has been examined afresh. This has been done both when the infant still had the dyspepsia in an unaltered state and when an improvement had taken place or transition to a normal condition had occurred.

In infants with a clinically mild form of so-called dyspepsia in breast-fed infants, the propulsive colonic motility has been less violent and often merely forced the contents forward a short distance at a less rapid speed, while in the medium form of the dyspepsia the motility was more violent, and in the severe form it was intense and often very rapid. The roentgenologic result was in agreement with the clinical picture of the dyspepsia.

No essential difference in the results as between the two methods of investigation could be established. Possibly the colonic contractions in the lighter cases of the dyspepsia were rather more violent when the contrast medium was applied as an enema rather than by mouth.

In a similar examination of *the normal infants* no movement in the colon was observed during feeding in the great majority of cases. On occasions a variation in haustration was noticeable, and in isolated cases a light propulsion which sometimes led to defecation, but of a gentle nature compared with the violent and often explosive defecation in the case of infants with the dyspepsia (*Plate 2*).

Transit time through colon. This varies very considerably in infants of this age. The frequency of defecation is of the order of 5 per day in the dyspeptic infants and 3, 2, 1 per day or less in normal infants. Soveri found that, in infants, the transit time of gas through the colon, which moves more rapidly than other contents, was one to two hours. Observations have been made on several occasions of how, in the dyspeptic infants, the contrast medium in the lower ileum, when reaching the ileocecal sphincter, starts to pass through it and continues through the colon to be then discharged. The time taken in complete transit and emptying has been between 4.35 minutes and 8 minutes in the cases observed. (Observation lasted at the most 10 to 12 minutes from the commencement of feeding.) In the cases where contrast medium was found in the colon, emptying of the complete contents of the colon at times took place in half a minute.

B. Experiments in feeding by different methods including the elimination of different factors in the reception of food — Simultaneous experiments with different types of nourishment

It has long been known that the so-called dyspepsia in breast-fed infants, as the term also implies, mainly occurs in breast-fed infants. For this reason there has been a desire to eliminate the factor which was believed to be of sole or main importance, namely the type of food, by introducing other nourishment as an addition to, or even as a complete substitute for, breast-milk.

In roentgen examination the changes in the colon described in Section A of this Chapter have appeared in the process of feeding. The time interval from the commencement of feeding to the start of the propulsive motility of the colon, and to pains, defecation etc, corroborates the assumption that feeding is an essential factor in bringing on the dyspepsia. Experiments have therefore been made with different methods of feeding, including the elimination of different factors in the reception of food, in order to judge their significance for the peristalsis in the dyspeptic and the normal infants. For this purpose breast-milk and cow's milk mixtures were used. The infants were given the nourishment they were accustomed to. The conditions for the experiments were standardized as far as possible, and I arranged for the infants to follow their normal feeding times and attempted to adapt the roentgen examinations to these times so that the infants should not come to an examination immediately after a meal. The same technic as described earlier was adopted. The investigation comprised the following tests: —

1. *Breast-feeding*

The investigated children that were accustomed to the breast were breast-fed. They exhibited, to a greater or less degree, a propulsive motility in the colon according to the severity of the dyspepsia. In the normal infants no colonic motility was observed, or at any rate only insignificant. The results are recorded in Section A of this Chapter.

2. *Bottle-feeding*

In this investigation the mother was not present (the sight and hearing, smell, taste and feel of the mother were eliminated). Tests

were made with different bottle contents, breast-milk, citric acid milk or half-milk. These tests were carried out on 15 infants. Of the 15, 2 were normal infants, and in their case feeding produced no peristalsis in the colon, nor defecation. In the remaining 13 infants a lively propulsive motility of the colon was obtained in 8 cases, leading to defecation in 5 cases. Attacks of pain developed in 5 of the infants. In 5 infants there was no reaction of propulsive colonic motility, but in one of them of a kneading movement in the colon. In 2 cases the experiments were repeated after treatment with Skopyl, but the lively colonic peristalsis experienced earlier could not be reproduced. New experiments were made after the treatment of 2 infants with Larosan, the same lively propulsive motility being produced as earlier. No difference in the appearance of the peristalsis was demonstrable after breast-milk or cow's milk mixture. Both breast-fed and artificially fed infants were found among those in which feeding started a colonic peristalsis and among those in which no colonic peristalsis was evident.

3. *Feeding by spoon*

The mother was not present. (Thus in this investigation sucking was also eliminated.) Altogether 8 infants were examined, of which 3 normal. Out of the 5 babies with the dyspepsia there was in 2 cases a light to medium propulsive motility of the colon during feeding, while in 2 others no colonic motility was demonstrable. The fifth infant, a violent case of the dyspepsia, vigorously resisted feeding, screamed, gurgled, twisted and turned, but despite this received 100 Gm. breast-milk in 6 minutes. This infant, 1.35 minute after commencement of feeding, developed a violent contraction in the cecum which was not followed by propulsion or defecation. Of the infants which had a peristaltic reaction of the colon, one defecated.

The record reads as follows: "5½ hours after contrast *per os*. Contrast in colon. Spoon-fed. 90 Gm. breast-milk given in 9 min. 1.05 min. after beginning of feed peristalsis observed in small intestine. After 1.20 min. peristalsis commences in ileum at ileocecal sphincter, which is clearly defined, approx. 3 mm. wide when contrast passes. At 1.40 min. a peristaltic wave occurs in cecum and about half of the medium separates as a sausage and moves forward quite gently down to rectum. When contrast is properly collected in ampulla, defecation occurs at 3.45 min. New wave at 4 min. carrying the remaining medium through colon; medium collects and is partly defecated at 7.10 min. Less than half of contrast left in colon, and

at 7.30 min. a last remaining portion begins to move down, reaching ampulla after same time as previously. Examination terminated 10 min. after commencement of feeding."

The other infant to exhibit a peristaltic reaction of the colon was the only one of the five to suffer pains during the examination.

The record was as follows: "7 hours after contrast *per os*. Contrast in colon. Spoon-fed with breast-milk. Peristalsis of small intestine commences. Gas discharged 3 min. after commencement of feed. At 4.30 min. patient in pain, refuses to eat, writhes etc. Contraction of sigmoid colon observed. Light to medium propulsive motility in colon apparent."

Of the normal cases, 2 exhibited no colonic motility during feeding, while the third, a 28 days old premature, of birth-weight 2 100 Gm., a borderline case to the dyspepsia, reacted by a lively kneading, non-propulsive movement in the colon.

Record: "7 hours after contrast by mouth. Breast-milk by spoon. Feed taken in 4½ min. Ate well. At 1.15 min. lively movement in small intestine observed. Lively kneading movement in colon gradually begins at about 2.30 min. Livelier haustration than normal. Substantial contraction but without propulsion occurs at 4 min. No defecation."

Only one of the dyspeptic infants received cow's milk mixture, and no peristaltic reaction in the colon was observed on examination.

4. *Feeding by tube*

The mother was not present. In this investigation no food comes into the mouth and esophagus, and moreover the factors of sucking and swallowing are eliminated. The investigation comprised 11 infants, of which 2 normal. No colonic motility was demonstrable in either of the latter, while 3 of the 9 dyspeptic infants exhibited a light to medium propulsive motility of the colon on feeding. Two of them defecated (*Plate 6*). None of the infants showed signs of pain in the course of the investigation. None of the remaining 6 infants exhibited propulsive motility of the colon, but one of them developed violent contractions in the colon of a non-propulsive character. Breast-milk was fed to 9 of the infants and citric acid milk to 2. One of the latter reacted with a medium propulsive motility of the colon followed by defecation.

5. *Feeding from washed breast*

In order to eliminate the taste and, to some extent, the smell of the mother to see whether the peristaltic conditions would be affected thereby, the breast of 3 nursing mothers was washed with alcohol and ether.

A while after the ether had evaporated the infant was put to the breast. As in normal feeding, a violent propulsive motility of the colon was provoked in 2 of the infants, followed by defecation but without pains. The peristalsis was perhaps rather less violent than on feeding from the unwashed breast. A merely insignificant peristalsis of the colon was demonstrable in the third infant.

6. *Sucking at empty breast*

In order to determine whether sucking per se in combination with intimate contact with the mother would be a sufficient stimulant to provoke a colonic peristalsis in the infants suffering from the dyspepsia, the following experiments were made. Cases were taken of mothers without breast-milk, whose babies were fed on other food, but despite this the baby was put to the breast. The main difficulty was encountered in getting the infant to take the breast, and therefore the investigation only comprises five cases. At least 14 days had passed since breast-feeding had been abandoned. In three infants with the so-called dyspepsia in a severe form sucking the empty breast provoked a violent propulsive motility in the colon, and in two of them defecation followed. None of the infants exhibited signs of pain in the course of the investigation, even if one of them cried almost continuously. One of the infants which had exhibited a peristaltic reaction, was put to the breast after the breast had been washed with alcohol and ether. Some time had by then elapsed so as not to tire the child. When sucking recommenced, no peristalsis in the colon was demonstrable. (The same infant had a lively reaction of propulsive motility of the colon when bottle-feeding took place with citric acid milk and moderate peristalsis when citric acid milk was given by tube.) Of the two remaining infants, one did not react at all when sucking, and the other very slightly.

7. *Sucking on nipple shield with unperforated teat*

This part of the investigation involved intimate contact with the mother (apart from the sense of taste) and the sucking element. Eight infants were put to suck on an unperforated teat placed on a nipple shield over the mother's breast. In one case (severe form of the dyspepsia) a violent propulsive motility of the colon was observed.

The record reads as follows: "Contrast medium per os 6½ hours earlier. Violent peristalsis in small intestine observed 1 min. from commencement

of sucking. 1½ min. from commencement of sucking a violent propulsive motility of colon starts. At 2 min. patient in pain and not sucking. This condition lasts 1 min. after which he starts sucking again, and at 4½ min. propulsive colonic motility returns."

In another case a mild propulsive motility of the colon was observed, and in a third a light to medium non-propulsive motility. The latter infant was for a while restless — 2.45 min. from the commencement of sucking — and writhed in apparent pain from contraction of the sigmoid colon. In the remaining 5 dyspeptic cases no colonic motility was observed in this part of the investigation nor sign of pain. No infant defecated in the course of investigation. Seven of the infants were breast-babies, while one, in which there was no colonic reaction, was a bottle-baby.

8. *Sucking from unperforated teat*

Here the sucking element alone was involved, and the mother was not present at the investigation. Five infants were put to suck on teats attached to empty bottles. In one severe case of the dyspepsia — the same as exhibited a lively propulsive motility in test 7 — a slight propulsive motility of the colon was observed and the infant suffered a slight attack of pain. In two cases a mild contractive, non-propulsive motility of the colon was observed (one being a bottle-baby), while in the remaining two cases (breast-babies) no motility of the colon was discovered. None of the infants defecated in the course of the investigation. Only one infant referred to reacted with pain.

9. *Intimate contact with the mother without sucking or feeding*

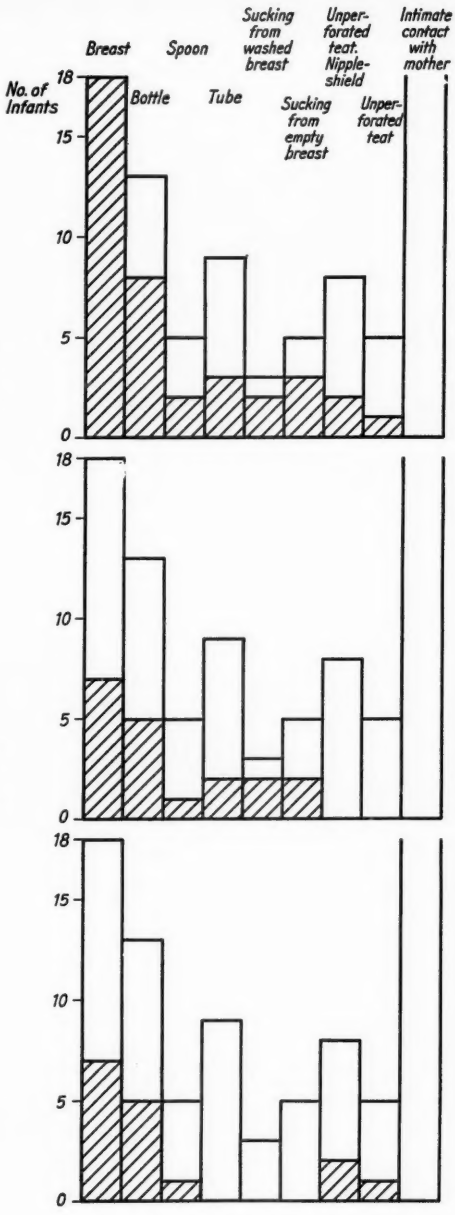
In a large number of cases the mother lay on the trochoscope and cuddled her child. The child lay at the mother's naked bosom but was not allowed to suck the breast. No motility of the intestine or defecation was provoked.

10. *The condition of the small intestine on sucking from an unperforated teat*

In those parts of this investigation in which food was given, peristalsis in the small intestine commenced and continued as described in Section A of this Chapter. In eight tests on altogether six infants, of which one normal, the motility of the small intestine during sucking

Fig. 4.

Experiments in feeding by different methods including the elimination of various factors in the reception of food, and the influence on colonic peristalsis, defecation and attacks of pain.



A. Propulsive colon motility

Shaded columns signify propulsive colon motility.
Unshaded columns signify no or insignificant propulsive colon motility.

B. Defecation

Shaded columns signify defecation during X-ray examination.
Unshaded columns signify no defecation.

C. Attacks of pain

Shaded columns signify attacks of pain during X-ray examination.
Unshaded columns signify no attacks.

alone was studied. The infants were put to suck on nipple shields with unperforated teats or on unperforated teats placed on empty bottles. In all cases a normal peristalsis of the small intestine occurred. It started about one minute after the commencement of sucking due to the fact that the contrast medium was far down in the small intestine and was in all cases administered several hours before the examination. On the other hand it was observed on two or three occasions that infants sucked their knuckles, but not intensively, at the commencement of the examination, no appreciable movement of the small intestine, however, being provoked thereby.

These experiments prove that in infants suffering from so-called dyspepsia in breast-fed infants the most violent propulsive motility of the colon is provoked by breast-feeding. This peristalsis may, however, be provoked by other forms of feeding as well. Bottle-feeding, whether with breast-milk or cow's milk mixture, produces next to breast-feeding the most violent and most constant reaction. In cases where food was given by tube or spoon, so eliminating the sucking element, peristalsis in the colon was produced less often and was less violent. But sucking alone in contact with the mother, and even sucking without contact with the mother, may also at times produce propulsive motility of the colon in infants suffering from the severe form of the dyspepsia. Mere contact with the mother, even when intimate, did not on the other hand produce the same results. The investigation is represented graphically in *Fig. 4*. The experiments show that there is in these infants an innate manner of reaction, the result being undoubtedly affected by numerous elements — the proximity of the mother (sight and hearing, smell, taste and feel of the mother), sucking, food in the mouth, esophagus, ventricle and duodenum, and swallowing. Several of these elements are individually capable of producing peristalsis in the colon, but the reflex is not generally so pronounced that sucking alone, without food, provokes motility; other factors as well such as feeding and swallowing are required for its provocation.

In these special investigations only a small number of normal infants have been examined. Reference may be made to Section A, where tests on normal infants under breast-feeding are described. It is hardly conceivable that an infant should develop propulsive motility of the colon by artificial feeding, but not breast-feeding.

CHAPTER V

Pressure measurement in the rectum

With a view to forming a rough idea of the pressure and changes of pressure in the rectum, an investigation was made comprising 16 infants, in 8 of which so-called dyspepsia in breast-fed infants was present, 5 others having had the dyspepsia and become normalized, and 3 being normal infants.

It is to be expected that, in the dyspeptic infants, the pressure in the colon and rectum will, at least on occasions, be heightened. A quite other resistance is encountered on administration of contrast enema, which is manifest both in the need of increased pressure in administering the enema and in the tight contraction of the colon as compared with the normal infants. The strong contractions occurring in the dyspeptic, but not in the normal, infants for propulsion of the contrast medium and for the violent discharge, probably indicates a higher pressure. Attempts have been made to measure the variations of pressure in the intestine by different methods. Good results are obtained by the most common procedure employing single and tandem balloons. Unfortunately the method cannot be used for the colon of the dyspeptic infants since the frequent contractions and discharges would either force out the balloon or give an erroneous and unphysiologic picture of the intestinal activity if the balloon remained in the intestine and obstructed propulsion. Instead a heart catheter was used which was placed about 4 cm inside the rectum and connected to a T y b j a e r g H a n s e n Universal Manometer.

Normal infants have, during feeding, shown a rectal pressure of up to 60 cm. water pressure. The differences of pressure in the case of normal infants have been small, have taken place without rapid changes and not exceeded 25 cm. water pressure. Infants, which have become normalized after having had the dyspepsia, differ from the normal infants in the greater changes of rectal pressure. Infants with the dyspepsia in a severe form exhibit a strikingly high pressure during feeding, the majority a maximum of over 100 cm. water pressure, and furthermore great pressure changes. One infant had a pressure differ-

ence of over 100 cm. water pressure. In five cases the pressure could be measured when the infants had an attack of pain — only in one case, however, were higher values obtained than during breast-feeding — but the pressure differences were high, as during defecation. The light and medium cases of the dyspepsia have shown lower values and smaller pressure differences than the severe cases. In three infants with the dyspepsia in a severe form, pressure measurements were repeated after treatment with Skopyl and lower values and smaller differences in pressure were then obtained. *Plate 8* records instances of the pressure curves obtained.

CHAPTER VI

Treatment Studies

Treatment of so-called dyspepsia in breast-fed infants is indicated more or less in accordance with the degree of severity. The light cases have not caused much concern as improvement has been rapid without treatment. The moderately severe cases have not seldom required special medical treatment, and this has applied even more so in the severer cases with their frequent stools, pains and neurolability. Treatment has differed according to the view held of the cause of the syndrome. A common treatment consists of an addition of boiled cow's milk or mixture, and was in fact being recommended 50 years ago by Raczynski and later by Finkelstein, by Czerny-Keller as an "allaitement mixte", and by others. The addition of a special protein compound has been recommended by, among others, Finkelstein, Rominger and Brennemann (as prophylaxis against "three months colic", possibly combined with some type of milk mixture). Marfan recommended belladonna, and several, among whom Jundell, Alarcon and others, have used atropine with good results, as did J. A. Anderson against colic. Marfan gave bismuth in addition to belladonna, Cathala recommended among other things calc. precipite, and Calzada Rodriguez atropine and phenobarbital with locally active agents such as tannin and bismuth.

The following experimental treatment has been undertaken in my investigation:

No. of infants treated with	So-called dyspepsia in breast-fed infants			Total
	Light	Medium	Severe	
Skopyl	4	16	39	59
Protein	—	3	4	7
Cow's milk or mixture	6	3	11	20
Change of environment	4	9	13	26
Untreated	4	10	6	20

The results of treatment have been judged clinically in respect to stools, attacks of pain and neurolability, and roentgenologically in respect of propulsive motility of the colon.

When normalization of the stools has been immediate (or in certain cases within 3—4 days) and been brought about by a small dose of Skopyl, it has been referred to as a *prompt effect.* The frequency of the stools has been taken into account — 3, 2, 1 or less a day — and to some degree their consistence and appearance. If a larger dose had to be administered to achieve the same result (immediately, or in certain cases within 3—4 days), it has been termed a *good effect.* A *moderate effect* has been referred to when normalization occurred in the course of the second week, or if the frequency and appearance of the stools appreciably improved, yet in spite of improvement could not be classified under the other headings. This basis of classification is that used for appraisal of the infants treated with Skopyl, and has also been employed for other forms of treatment. The same classification has been applied in regard to the influence of pains and of the neurolabile symptoms which can be affected by treatment (see under treatment with Skopyl). As regards roentgenologic appraisal the same designations have been employed as above, a prompt effect being considered as complete normalization of the colonic peristalsis at the first, or possibly subsequent, roentgen examination after the commencement of treatment (see *Table 12,* p. 58); a good effects as almost complete normalization, e. g. from a violent to a light propulsive motility in the colon; and a moderate effect as a decided improvement. As regards infants treated by change of environment and untreated infants, a good effect (i. e. a good improvement in untreated cases) has been considered as normalization of the respective symptoms within a week; a moderate effect (i. e. moderate improvement in untreated) as a distinct improvement in the respective symptoms during the first week or complete normalization during the second week after the commencement of treatment or observation.

1. Skopyl treatment

Skopyl, Methylscopolamine nitrate, is manufactured on the lines proposed by Docent E. Nyman of the Swedish pharmaceutical firm. AB Pharmacia. Skopyl is an anticholinergically active preparation

significantly superior to atropine as a vegetative spasmolytic. Its antispasmodic action is more than five times stronger than that of atropine on the intestines of guinea pigs (Nyman).

In 1944 treatment with Skopyl was commenced at the Samariten Children's Hospital on infants with pylorostenosis. In the same year I started treating cases of so-called dyspepsia in breast-fed infants with this preparation. The working hypothesis was that the symptom complex was due to a lack of balance in the vegetative nervous system with an increased tonus and a hyperperistalsis in the intestine. If this hypothesis was correct, an anticholinergically active formula should be beneficial. An alcoholic solution of Skopyl was given sublingually in drops (0,05 mg Methylscopolamine nitrate per drop) 20—30 minutes before each meal, that is 5—6 times a day. On the first three days the infant had as a rule received one drop on each occasion, and if the desired result was not attained, the dose was increased to two drops and later possibly 3 drops × 5—6. The latter dose has on only one occasion been observed to produce mild and rapidly passing signs of overdosing in the form of restlessness and pinkness in the face. The result of treatment was striking.

Clinically the stools were often immediately normalized. In 40 cases out of 61 the reversal came after a small dose (1 drop × 5), i. e. a prompt effect. In 14 cases a larger dose had to be administered to obtain the same result, a good effect. In 7 infants the effect was moderate. *Table 10* presents the results obtained with 59 infants. Two of them, which were not fully normal after a short period of treatment, have been included also after exhibiting normal conditions on renewed examination. Generally speaking, the time at which improvement took place gives a good picture of the action of the medicine, since a prompt effect to a great degree eliminates the possibility of improvement having taken place without medicine being administered. As regards this possibility, the time of administration also plays a significant role. If the administration comes late in the process, there is a greater chance of spontaneous improvement. In 28 cases the administration took place in the first month of life, and in 28 in the second month. The pains have also been influenced by the therapy; they have decreased or disappeared, though often only on administration of the larger dose (32 prompt effects, 15 good effects). The tendency to vomiting decreases or disappears. The influence on neurolabile symp-

Table 10. Results of Treatment

	Prompt effect	Good effect	Moderate effect	No or poor effect
A. Effect on stools and propulsive colonic motility				
1. *Treatment with Skopyl*				
Clinical appraisal	40	14	7	—
Roentgen examination	36	4	3	2
2 a. *Treatment with Protein*				
Clinical appraisal	—	—	2	9
Roentgen examination	—	—	—	10
2 b. *Treatment with Cow's Milk or Cow's Milk Mixture*				
Clinical appraisal	—	—	—	20
Roentgen examination	—	—	—	14
3. *Treatment by Change of Environment*				
Clinical appraisal	—	—	3	23
Roentgen examination	—	1	—	13
4. *No Treatment*				
Clinical appraisal	—	—	3	17
Roentgen examination	—	2	1	12
B. Effect on pains (Clinical appraisal)				
1. *Treatment with Skopyl*	32	15	7	2
2 a. *Treatment with Protein*	—	—	—	11
2 b. *Treatment with Cow's Milk or Cow's Milk Mixture*	—	—	—	19
3. *Treatment by Change of Environment*	—	1	8	13
4. *No Treatment*	—	2	—	14
C. Effect on neurolabile symptoms				
1. *Treatment with Skopyl*	4	12	4	2
2 a. *Treatment with Protein*	—	—	1	10
2 b. *Treatment with Cow's Milk or Cow's Milk Mixture*	—	—	—	15
3. *Treatment by Change of Environment*	—	3	12	6
4. *No Treatment*	—	—	1	15

toms, which have often been spontaneously reported by mothers, is more difficult to appraise and to distinguish from the aforementioned results of treatment. Previously restless and whiny infants have become quieter, generally do not disturb at night-time as before, sleep better, eat more peacefully etc., while no particular change in their sensitivity to noise and light has been noticed. In the infants where the result (see *Table 10*) could be determined with certainty, the effect on the influenceable neurolabile symptoms has in 4 cases been prompt, in 12 good, in 4 moderate, and in 2 slight or none.

The duration of treatment with Skopyl has been dependent on the intensity and development of the case. In comparatively mild cases, where a prompt effect was obtained, the administration could be stopped after a week. In more severe cases the dose was reduced after one or two weeks, and if improvement took place the administration was stopped a few days later. In the most severe cases treatment lasted longer, and if the Skopyl administration was experimentally stopped at an early stage the symptoms often returned immediately. Sometimes the records of the duration of the treatment have been dubious, but the results on 50 infants whose records are reliable are shown in *Table 11*.

Roentgenologically the results agree well with those obtained clinically. If the clinical result was good, the majority of patients show a picture resembling the normal condition with an easily filled out colon of normal width; no peristalsis, or only slight, during feeding; no pains and very seldom any defecation during examination. This is clearly shown in *Plates 3, a—b* before treatment and *c—d* after treatment. The roentgen picture after Skopyl treatment is also shown in *Plates 7 c—d*. If the result of treatment has been unsatisfactory clinically,

Table 11. Duration of Treatment

	Treatment			
	Skopyl	Protein	Cow's Milk	Change of Environment
1 week	6	3	1	2
8—15 days	16	6	1	14
16—30 days	17	2	7	9
More than one month	7		6	4
More than two months	4		2	1

Table 12. Time of Roentgen Examination after Commencement of Treatment

(The time elapsing from commencement of treatment or observation until roentgen examination when appraisal was made)

	Treatment				
	Skopyl	Protein	Cow's Milk	Change of Environment	No Treatment
1— 7 days	20	3	1	7	8
8—15 days	18	5	3	4	4
16—30 days	6	2	6	2	4
More than 30 days	1		4	1	1

hyperperistalsis of the colon is still apparent roentgenologically. Generally the roentgen study of the infant took place a week subsequent to the roentgenologic diagnosis of the dyspepsia and commencement of treatment. At times, however, the examination was not made until the second week, usually because only a limited number of infants could be dealt with by the Roentgenologic Department (cf. *Table 12*). A roentgenologic study was made of 40 infants both before and after treatment. In 4 cases in which the result was not adequate after a short period of medication, a new roentgen study was made within 14 days, a normal picture then appearing in all cases. These cases are recorded in the Tables both from the first and second examinations, as also of one infant which had a long relapse when Skopyl was stopped and showed a normal picture when the drops were given again. No patient with mild dyspepsia is included in the 40 cases.

The pressure conditions in the rectum of three patients were examined before and after Skopyl medication. After treatment they exhibited lower pressure and smaller pressure differences.

2. Treatment with protein, cow's milk or cow's milk mixture

Since therapy consisting of protein or cow's milk is still widely used and recommended, tests have been made on these lines. Seven infants were treated daily with 10 Gm. of a special protein compound (calcium caseinate), five with Laktén (Astra), two with Larosan (Roche). Twenty infants were treated with purely cow's milk mixture or minimum of 50 Gm. cow's milk added daily to breast-milk. The cow's milk

was generally given in the form of citric acid milk or as a half-mixture of half milk and half water-sugar-flour.

Clinically, the stools occasionally became firmer, but the frequent discharges and pains persisted unchanged, as did the neurolabile symptoms. Of 15 of these infants on a cow's milk diet and 4 on protein, treatment started in the first month of life. Seven infants were treated with protein, and four of them underwent more than one observation. Records of both the first and second examination of these cases are partly found in the Tables. The results of treatment are recorded in *Table 10* and the duration in *Table 11.*

Roentgenologically 14 infants treated with cow's milk mixture and 7 treated with protein were examined (3 of the latter by double observation — 2 being re-examined after 14 and 21 days of continued treatment respectively, and 1 being later treated afresh and re-examined thereafter). In all of the infants roentgen examination revealed, in spite of treatment, a more or less violent propulsive motility of the colon on feeding. This motility of the colon in infants treated with protein corresponded to that occurring on roentgen examination before treatment started. Since infants treated with cow's milk mixture had, with one exception, had their mixture when they came for examination, roentgen studies were not conducted until treatment had been proceeding at least a week, and usually much longer (*Table 12*). In these infants a propulsive motility of the colon appeared on feeding, and the roentgenologic changes were in agreement with the clinical findings and the clinical classification. Treatment with protein continued in one case 5 days before a roentgen study was made, but a new study after 14 days' treatment still revealed no change since the start of treatment. In the other cases on protein, treatment continued between 7 and 28 days before the roentgen examination, and in no case was an appreciable improvement demonstrable in relation to the examination before treatment (cf. *Table 10*). *Plates 7 a—b* show a case after treatment with protein, and *Plates 7 c—d* the same case after treatment with Skopyl.

3. Treatment by change of environment

Since a large part of the infants' disorders are typical neurolabile symptoms which may have been aggravated by the commonly nervous environment they live in, a change of environment should exercise a

favorable influence on so-called dyspepsia in breast-fed infants. Some infants were therefore taken into the Samariten Children's Hospital so as to make it possible to follow the influence of hospitalization both on their gastro-intestinal activity and their neurolabile symptoms. An important reason for hospitalization of some of the infants was the severe symptoms of restlessness, sensitivity, crying — particularly at night — and poor sleep, which in a number of cases had made life intolerable to the mother and other members of the family. No change apart from that of environment took place. The mother came to nurse her child, and where the infant was accustomed to other food, this was given in the same way as before hospitalization. Altogether 26 infants were treated in this way. Four of them are represented in the Tables by double observations, and one with three observations. In the group of dyspepsia in severe form were 13 infants. In the case of 11 infants treatment started in the first month of life.

Clinically, the influence on the frequency, consistence and appearance of stools was in most cases slight. The effect on pains was better (good effect on 1 infant, moderate effect on 8), though the difference in observation between home and hospital should possibly be taken into account. After nursing the infants were put to bed in the hospital and left alone, while at home the mother often watched anxiously over every sound from the baby. Most interesting is the frequently good influence on the infants' neurolabile symptoms. Most of the infants became by day less fretful, and less restless and anxious. Their excessive sensitivity, particularly to noise, remained, but they did not give way to the long uncontrollable attacks of screaming they had at the beginning of hospitalization. The motor restlessness decreased, and sooner or later most infants became quiet at night. To start with, they perhaps did not sleep the whole night, but generally lay quiet when awake and soon slept the whole night through. Among the infants investigated, however, were some which did not show any improvement in their nervous reactions either. They had to be put in separate rooms to avoid disturbing the other infants by their daily and nightly screaming, but again treatment with Skopyl had a beneficial effect. The results of the investigation are recorded in *Table 10,* showing that a good effect was obtained in 3 infants, and a moderate effect in 12. *Tables 11 and 12* show the duration of treatment and the time from starting treatment to later examination by roentgen.

The results suggest that, apart from the neurolabile disposition of the infant, the influence of environment is often significant, and a change in this respect produced a beneficial effect. In the cases where favorable results were attained only when treatment was implemented by the administration of Skopyl, it appears likely that the gastro-intestinal disorders were of such intensity that the infant could not benefit by the influence of the restful environment until the disorders had diminished or disappeared. It should be pointed out, however, as has been asserted earlier, that many of the infants underwent a rapid improvement as regards certain of their neurolabile symptoms as a result of Skopyl medication alone without any change of environment.

Rocntgenologically 8 infants were investigated both before and after treatment, and 6 when treatment had been proceeding for a longer or shorter period (*Table 12*). No pronounced difference, except in one case, was observed in the propulsive motility of the colon in connection with feeding as between roentgen studies before and after treatment. In these seven cases, and in the other six, roentgen examination after treatment showed the same severity as did the clinical picture and the clinical classification (cf. *Table 10*).

4. No treatment

To implement the groups above, an investigation was made of 20 infants (of which 3 are partly represented in the Tables by double observations) without making any change as regards feeding or environment. No medicine was given. A comparison with the previous groups must be of value, since even without treatment a normalization of the intestinal activity occurs sooner or later. The time of normalization depends on the age and the severity of the dyspepsia. If an untreated infant is examined immediately before its stools become normal, proof may often be obtained clinically and roentgenologically that the gastro-intestinal symptoms are on the wane, and on renewed examination after a week or more a more or less normal picture is found. In other cases no change of the complex of symptoms comes to light on renewed examination.

Clinically the normalization of stools has occurred in the light cases at an age of about one month, and in the other groups from about one month up to six months. On the basis of the method of appraisal

described above, in 3 infants the improvement was moderate, while in 17 there was no improvement or only slight.

Roentgenologically 17 infants underwent a complete examination, i. e. a second roentgen examination following a week or more after the first. In two infants improvement was good (one of which, however, was over 6 months old), in one there was moderate improvement and in twelve none. The remaining two infants which had the dyspepsia in a moderate form came to examination 30 and 37 days respectively after the first examination, and should from a clinical view have normal stools and did in fact show a normal picture clinically and roentgenologically. In *Table 10* recording the roentgenologic results, the two latter infants are excluded.

To summarize, it may be said that as regards the infants' intestinal symptoms the results of treatment with protein, cow's milk mixture and change of environment were similar to those of the untreated infants. Individual infants have thus improved to a greater or less degree within the two weeks I set as the limit for judging the result of treatment, while the great majority have remained unchanged clinically and roentgenologically. The difference in those treated with Skopyl, whose intestinal activity became rapidly normal, is striking. The infants' pains also are quickly affected by Skopyl medication, and to some extent by change of environment, but not at all by other forms of treatment. The neurolabile symptoms of the infants have been affected beneficially both by Skopyl and by change of environment, while no manifest change was brought about by other forms of treatment nor in the untreated infants.

CHAPTER VII

Discussion

A. Peristalsis

Among the problems met in so-called dyspepsia in breast-fed infants, those connected with intestinal peristalsis become evident at an early stage and are of fundamental importance. In this Section, and to some extent in the next, a discussion will be made of the gastro-intestinal symptoms and of the physiology of the bowel movements and kindred problems, but not of such hormonal or other factors which appear in the new literature without appearing to have any direct influence on the dyspepsia.

1. Peristalsis of the small intestine

It was demonstrated in Chapter IV how peristalsis of the small intestine was induced by feeding, both in the normal and the dyspeptic infants. Even the mere sucking on a teat was sufficient to provoke this peristalsis. It commenced in the duodenum about 15 seconds after the beginning of feeding and spread rapidly to the other parts of the small intestine. It was seen how one quantity of contrast medium after another was reached by the lively peristalsis, and finally how even the more concentrated portion of the contrast medium in the lower ileum was set in motion after about 1½ minutes. The speed of the undulating movement is in agreement with the speeds of 2—10 cm per second recorded in the literature. The time of transit of the contrast medium through the small intestine varied greatly both in the normal and the dyspeptic infants, in round figures between 2 and 7 hours (cf. Fig. 1, p. 39). This large variation, which also was found by Henderson (1942) in newborn and by Lönnerblad (1951) in one-year-old normal infants, means that there is no clear dividing line from the pathologic, i. e. too rapid or too slow, transit time. Calzada Rodriguez subjected 20 infants with prandial diarrhea to radiologic examination and found "la traversée digestive du lait" hurried, at the same time demonstrating a passing spasm of the small intestine.

He recorded no comparative material and stated a transit time through the small intestine of 4—6 hours to be accelerated, a transit time which according to my investigations and those of Henderson and Lönnerblad is not pathologic. In the present material investigated, no difference has appeared in the small intestine as between the normal and the dyspeptic infants.

According to Golden there are certain differences in the physiology of the intestine of the young infant and of the adult. Bouslog believes that the differences lie in the anatomic structure of the intestinal wall, while Golden points out that nervous control, in general, is far from completely developed in the newborn.

2. Peristalsis of the colon. Higher centers for intestinal movements

In infants suffering from the dyspepsia a violent propulsive motility of the colon occurs about 1½ minutes or more after the commencement of feeding, which often practically empties the colon and usually the rectum too. Sometimes a new peristaltic wave comes quite soon after emptying of the rectum, an interval of one minute between the movements being not unusual. The same thing was found by Weitz and Vollers who noticed in a man with a very thin anterior abdominal wall that the colon contracted once a minute. In the normal infants there was only insignificant or no colonic motility on feeding.

The rapid movements that take place in the transport of the colon contents, so-called mass peristalsis, were described by, among others, Holzknecht (1909): cf. also the following Section on the ileocecal sphincter and gastro-colic reflex.

After vagotomy in man there is sometimes a tendency to diarrhea, which according to Alvarez may be due to an increased irritability of the bowel. Learmonth and Markowitz, among others, have shown that increased colonic activity follows section of the sympathetic nerve fibers to the colon. Best and Taylor maintain that section of the pelvic nerves (cholinergic nerves) relaxes the wall of the distal colon, and that "section of the cord above the sacral segments also causes relaxation, which indicates that the constant augmentor effect is due to impulses arising in higher centers". Youmans found in dogs that denervated segments of jejunum were more excitable and responded more violently to distention. An increased motor activity

in the colon has been obtained in experiments with parasympathomimetic agents (by among others Grace et al., cf. Section G on treatment), while anticholinergic drugs reduce this activity. — Thus by various means the influence on the bowel by the higher nerve centers has been disturbed, which in a parasympathetic activity has resulted in a hyperactive colon. An altered function has also been obtained by degenerative section of all the extrinsic nerves of the bowel, and according to Alvarez these animals often die of diarrhea and inanition. The dependence of the bowel on higher centers has also been demonstrated in other ways, by Ström and Uvnäs (1950) among others, who found that hypothalamic stimulation in cats yielded augmentatory as well as inhibitory responses. They obtained increase of tone and vigorous peristalsis in the duodenum and jejunum, while they more seldom obtained an effect on the ileum. Response was also obtained from the colon. A post-stimulatory period of increased activity in the small intestine and colon was often observed. They further obtained motor responses from the gastro-intestinal tract on stimulation of the cortex and the basal ganglia. From the frontal lobe cortex predominantly inhibitory responses were evoked, from the basal ganglia predominantly augmentatory. Watts and Fulton (1934) found that electrical stimulation or extirpation of the premotor cortex in monkeys produced an increased gastro-intestinal activity.

The propulsive motility of the colon demonstrated *in the dyspepsia* corresponds to that obtained in different ways by a disturbed inhibition or control of the central nervous system with a parasympathetic predominance. Nervous control in newborn babies is far from being completely developed. It is therefore conceivable that so-called dyspepsia in breast-fed infants is due to the failure of the vegetative higher brain centers (perhaps particularly in the undeveloped cortex) to exercise their inhibitory and controlling function, the ability to do so being developed later. This theory would accord with the fact that the infants' hyperperistalsis diminishes and disappears within one or a few months. If the dyspepsia were due to arrested development, it should be found to be more pronounced and more frequent in *premature babies.* In the present investigation there were 5 infants with a birth-weight of between 1 500 and 2 500 Gm. In addition there was one infant suffering from debility which was born three weeks too early but weighed 2 720 Gm. The latter infant and one of the others

exhibited a moderate dyspepsia, and a third a severe dyspepsia, while the remaining three were placed in the normal group; two of them, however, were on the border-line to the dyspepsia. One had normalized stools at an age of 3 weeks, becoming frequent again when the infant was put to the breast, and exhibited a lively, kneading, non-propulsive motility in the colon on spoon feeding at an age of 4 weeks. The other infant had frequent stools up to about 3 weeks of age, when the frequency dropped, and at an age of 1 month a colonic movement was seen under roentgen examination, though within normal limits. These were the only 6 infants suffering from debility to be investigated, and therefore no pronouncement can be made on the frequency of the dyspepsia in premature compared with full-term infants. If there is a predominance of the dyspepsia in prematures, it appears likely that it is due to the adaptation referred to earlier, which may take longer in the premature infants, while in the dyspeptic children the neurolability is presumably the decisive factor. It is not to be expected that neurolability will be more common in premature than in full-term infants.

The rectum possesses a high degree of automaticity, but in addition there is in the medulla a center for defecation. The mechanism of defecation, when there is enough matter in the colon, is considered primarily dependent on the reaction of the rectum to distention. Hertz maintains that a few times a day there are "mass movements" which carry material usually from the transverse colon over into the sigmoid region. These movements may give rise to a call to defecation. In *infants with the dyspepsia* the colonic activity leads to defecation, which commences about 6 minutes after the beginning of feeding. The material is sometimes carried by the mass peristalsis through the colon to ejection from the rectum all at once, sometimes in stages. The material sometimes collects in the rectum and is defecated on a new contraction, but sometimes recedes, often being ejected by a new wave. In these infants defecation is almost completely associated with the mealtime or the period immediately following, and is a stage in the propulsive intestinal motility described above.

The activity of the bowels is mainly governed by the intrinsic and the extrinsic nervous system. *Psychic factors* may influence the bowel activity via vegetative centers in the midbrain which on the one side are in contact with the cerebral cortex and on the other with neighboring emotional centers. Cannon (1911) found that any sign of

rage, distress or mere anxiety in a cat was accompanied by a cessation of peristaltic movements in the stomach and intestine. He said also that many emotional states are undoubtedly strong stimuli to peristalsis but it is equally true that other emotional states inhibit peristalsis. Thomas (1949) points out the domination of the autonomic system by the hypothalamus and the intimate functional relation between hypothalamus and neocortex. Yaskin (1943) writes: "The principal relay station for emotional components of diseases appears to be in the diencephalon. It is responsible for the correlation of psychic and somatic disorders, has a regulating influence upon both of the major divisions of the vegetative nervous system and indirectly upon most of the endocrine glands, upon metabolism and heat regulation." Grace, Wolf and Wolff found in 1950—1951 on fistulous subjects that the colon participated in reactions to stressful situations. They found that "overwhelming situations provocative of abject fear and dejection were associated with hypofunction of the large intestine with pallor, relaxation, lack of contractile activity and relatively low concentration of lysozyme in the colonic secretion. Stressful life situations provocative of conflict, anger, resentment and hostility or anxiety and apprehension were found to be associated with hyperfunction of the colon manifested by hyperemia, hypermotility and increased secretion of the enzyme, lysozyme."

Infants with the dyspepsia have on examination sometimes had severe crying fits, been tense and have resisted attempts at feeding. Once they have commenced feeding, they have become quieter, but some have continued to cry and struggle, and individual cases did not respond by propulsive motility of the colon in spite of receiving food, a condition which may be due to their disturbed emotional state. Neither in the dyspeptic infants nor in normal infants has any peristalsis been induced by crying (sometimes provoked by pinching the leg) in spite of increased abdominal pressure. At times of psychic stress the children often develop disorders of gastro-intestinal origin later on, when they are older, in the form of pains of the spasmus coli type. The parents and siblings also, more frequently than those of the normal infants, suffer from "irritable colon" or other "nervous" gastro-intestinal disorders, indicating that the disorders are due to genetic factors.

3. The ileocecal sphincter. Gastro-ileac or gastro-colic reflex

Alvarez maintains that one of the most important functions of the ileocecal sphincter and of the thickened muscle in the bowel just orad to it is probably to stop the peristaltic rushes. He continues, "I think it fortunate for us human beings that so many of the waves are thus stopped, because otherwise we might suffer with diarrhea ... The mode of control of the sphincter seems to be something like that of the pylorus, where irritation of the bowel about it or caudad to it causes material coming down from above to be held back. A receptive relaxation of the colon", he continues, "has been described as taking place when material is about to come through from the ileum. Destruction of the spinal cord or section of the splanchnic nerves causes a marked loss of tonicity in the sphincter with loss of function." Hinrichsen and Ivy found that stimulation of the vagus nerve may produce relaxation followed by contraction of the sphincter. They also found that the vagus nerve contains both motor and inhibitory fibers for the ileocecal sphincter. Stimulation of the sympathetic nerves or the injection of epinephrine usually causes contraction of the sphincter, whereas it causes relaxation almost everywhere else in the tract. They further found that reflex contraction of the sphincter may be brought about through either the extrinsic or intrinsic nervous mechanism.

In Chapter IV A was described how the peristalsis *in infants with the dyspepsia* was followed from its start in the duodenum, proceeding as a wave through the small intestine, driving the contents of the lower ileum through the ileocecal sphincter (which then appeared as a 2—5 mm wide connection between small intestine and colon), continuing as a strongly propulsive motility of the colon and ending up with a contraction of the rectum and defecation. All this takes place within a period of 6—7 minutes from the beginning of feeding.

The problem of regulation of the tonus of the ileocecal sphincter does not appear to be conclusively solved, but it seems as if parasympathetic nerve impulses would relax the sphincter. In the dyspepsia are found the same symptoms as are obtained in a parasympathetic predominance in the colon. That the sphincter opens and does not function as a "wave-breaker" between the peristalsis of the small and large intestines, as in normal infants, may probably also be explained as a parasympathetic predominance. This provides a natural explanation for the transition

of the bowel function to that of normal infants, as this predominance sooner or later becomes inhibited or controlled by higher centers. This explanation moreover makes it easier to imagine *the dyspeptic disturbances as a stage in a neurolability which appears to be particularly associatcd with the vegetative nervous system serving the gastro-intestinal tract.* Whether other expressions of a vegetative nature also occur is less easy to demonstrate, apart from the often observed sweating in these infants.

Hinrichsen and Ivy (1931) studied the ileocecal sphincter in dogs. They found that, if the lower ileum was filled with fluid containing barium and the animal was then given a meal, the last segment usually became very active. The sphincter relaxed and opened before an advancing wave of contraction in the small bowel, and within 15 minutes after the animal had started to eat, the lower end of the ileum was almost empty. They found that the gastro-ileac reflex did not appear to be a psychic one, because neither the sight nor the smell of food was ever sufficient to activate the mechanism. Even the giving of a small amount of food was insufficient to start the reflex. The response was most easily obtained when the animal was hungry and was not obtainable when the animal had been recently fed. Mac Ewen (1904) was the first to describe this reflex. Hertz (1909) recorded the reflex and named it gastro-colic reflex. He found that the chief stimulus to this movement is entry of food into the empty stomach, but in one subject he and Newton found that the movements occurred when the patient felt faint with hunger and food was brought into the room. Welch and Plant (1926) wanted to change the name to feeding reflex after they found that the taking of food produced activity in the colon of dogs and humans, while they never obtained this movement when food was given by gastrostomy. Douglas and Mann on the other hand found that a gastro-colic reflex in dogs could be started by entry of food through a gastric fistula within 1 to 7½ minutes from the beginning of feeding and that bilateral vagotomy did not affect the result. Friedman and Snape observed that the sight or smell of food produced a reddening and engorgement of the colonic mucosa in children with colostomy. Alvarez describes a man with an incompetent anal sphincter in whom the sight, smell or even the thought of food initiated bowel movement. Cannon found a close connection between ingestion and defecation and did not con-

sider he could distinguish between gastro-colic reflex and feeding reflex. Ivy found that the gastro-colic reflex, at least in dogs, was in fact a duodeno-colonic reflex, for the stimulation of the duodenum and upper jejunum caused defecation, while distention of the stomach never had such an effect.

In treatment of the dyspepsia with atropine or other anticholinergic agents, the fundamental cause is conceived as a vagotonia. This view was represented, for instance, by Jundell. The good results have provided support for the assumption that a vagotonia exists, cf. Alarcon and Calzada Rodriguez. The latter, as will be remembered, made a roentgen study of the intestinal tract and came to the conclusion that exaggeration of the gastro-colic reflex is the chief mechanism in the production of prandial diarrhea. Marfan considers the cause to be an intensification partly of the duodeno-biliary reflex with increased peristalsis of the small intestine, and partly of the gastro-colic reflex with increased colonic peristalsis and defecations at mealtimes.

In Chapter IV B an account has been given of attempts to eliminate various elements in the taking of food and the resulting influence on the propulsive motility of the colon. I found in *infants with the dyspepsia* that all developed a propulsive motility of the colon on breast-feeding, while the majority on bottle-feeding and less than half on feeding by spoon or tube reacted with colonic peristalsis (cf. Fig. 4, p. 49). Even the sucking on a teat without feeding resulted, in isolated cases, in colonic peristalsis. But no infant developed motility either in the small or large bowel when the mother cuddled it at her bare breast. Not even in these sensitive infants were purely psychic stimuli sufficient to initiate colonic peristalsis. When Hertz and Newton, Alvarez and others obtained bowel motility in adults on the sight and smell of food, this may be conceived to be the result of a conditioned reflex. In my investigation the mere introduction of a tube did not produce motility. The significance of sucking was clearly demonstrated. It was possible to establish that several of the elements forming part of the feeding process may independently be sufficient stimuli to initiate a colonic peristalsis, but generally several of these elements are required. These investigations have demonstrated an *important factor — the factor which activates the local mechanism* in so-called dyspepsia in

breast-fed infants — namely ingestion especially by sucking, and further provide an explanation why the complex of symptoms is not so common in infants fed by other means than the breast.

B. Symptoms from the gastro-intestinal tract

1. Stools

Diarrhea may be defined as the evacuation of more frequent and loose stools than normal, due to a livelier peristalsis than normal and an increased amount of fluid in the feces. One of the most important functions of the colon is to absorb fluid from the bowel contents, and its capacity in this respect depends largely on the rapidity of the passage and the degree of mucous secretion (Bonoff). Diarrhea may be caused by many things such as infections, bacterial and virogenic, often of an epidemic nature in newborn; digestive deficiencies, e. g. pancreatic insufficiency; nutritional deficiencies, e. g. of certain types of Vitamin B; endocrine disorders, e. g., hyperthyroidism; allergy; and finally functional disturbances — to name the most important (apart from a laxative diet and medicaments).

Increased peristalsis may broadly be considered as due to the following: —

a. *Central influence.* The function of the bowel is regulated by higher centers. The latter may be influenced by mental stress. Pituitrin and pitressin act on the bowel and cause, according to Puestow (1938), vigorous contractions of the colon ascribed to action on the smooth muscles, but he found that they have an inhibitory influence on the motility of the small bowel.

b. *Local influence.* The motility of the intestine is excited by its contents which stimulate mechanically and chemically. The chief chemical excitants are bile salts, organic acids and products of bacterial action (Ivy). Hecht and others have demonstrated that there are more volatile fatty acids in the stools of breast-fed infants than in those of infants fed on cow's milk mixture, and that these acids have a special excitative effect on the peristalsis and secretion of mucous membrane. Raczynski found more free fatty acids in dyspeptic than in normal infants breast-fed by the same nurse. Ivy further maintains, as experienced by many, that an increase in fat in the diet

of the infant or excessive carbohydrate shortens passage time because of the stimulating action on motility of the organic acids produced.

Alvarez refers to a "gradient", a gradual reduction in metabolic activity of the intestinal muscle from the duodenum to ileum with a downward gradation in the activity, tonus, irritability and rate of rhythmic contraction. He also asserts that there appears to be *a gradient in the pH* of the nervous tissues from duodenum to ileum, and in many animals and in man (Bucher et al.) there is a gradient in the pH of intestinal contents, with low values in the duodenum and high ones in the terminal ileum. Short has described attempts to alter the function of the ileocecal sphincter by putting either acid or alkali into the cecum without thereby altering the emptying of the ileum. Perger (1920) and Borchardt (1926) have demonstrated on animals that with a lower pH in the gastro-intestinal contents there follows a reduction of speed in the movement of the contents through the stomach and small intestine.

The feces of breast-fed babies exhibit a low pH — according to Freudenberg and Heller 5—5.4 and Nelson 4.7—5.1 — while infants fed on cow's milk mixture generally have stools which approach a pH of 7 or higher. Marfan mentions that infants with the dyspepsia have even more acid stools than normal infants.

Freudenberg and Heller found, on adding pure albumin to human milk, that in the great majority of cases a deterioration occurred in the consistence of the stools (in a dyspeptic direction) with at the same time a change in the bacterial flora. The addition of a protein-calcium compound, on the other hand, accompanied by an alteration of the reaction towards alkalinity and a changed bacterial flora produced an increase in firmness which was clearly demonstrable on the addition of finely powdered chalk. Czerny and Keller maintain that, if infants are fed for a considerable period on cow's milk without other addition than water, they almost invariably have dry feces due to the longer stay in the bowel, and this is again a result of decreased excitation of peristalsis.

In the dyspepsia the stools are thin and sometimes almost completely liquid. They are generally granular and the grains consist, among other things, of fat and fatty acids. In breast-fed infants, that is infants on identical feeding, it is noticeable that quite a large number have frequent stools (dyspeptic infants) and the majority have 1, 2 or 3 stools a day,

but that there are normal infants with an interval of a week or more between stools. Raczynski and others have shown that a woman can simultaneously nurse a baby with frequent stools and one with longer intervals. It is conceivable that this differing bowel activity occurs because some babies' intestines are more sensitive to the irritation of the contents or because of a deficiency in central regulation. *In the so-called dyspepsia in breast-fed infants the intestinal reaction follows so rapidly upon ingestion, being also capable of provocation without food, that a direct connection between the excitation by the bowel content and the propulsive motility of the colon is inconceivable.* Soon after the beginning of feeding, the rapid passage through the colon is initiated, and due to the short stay of the bowel content, particularly in the proximal part of the colon where the bulk of the fluid resorption takes place, the fluid resorption is appreciably impeded, which explains why these infants have loose stools. But they presumably also have *a locally sensitive bowel,* which is evidenced by the colonic reaction being more pronounced when the contrast medium is given by enema than by mouth, and by the colonic reaction which occurs in dyspeptic but not in normal infants when contrast medium is given by enema. They often react to additions to the food, and have difficulty in tolerating the novelty of weaning, they vomit more readily than other infants, and at a later stage they have a greater tendency to diarrhea, and are more sensitive to laxatives etc. It is conceivable that this sensitivity, as also the other intestinal symptoms, may be explained by increased central nervous impulses (cf. Ström and Uvnäs, p. 65) and by the consequent facilitation.

The consistence of feces is influenced, as said, both by the fluid content, which is mostly dependent on the time the material remains in the bowel, and by the components and their water-binding capacity and viscosity etc. In infants with the dyspepsia it is the rapid passage through the colon which is the essential factor, and which, even in an infant with this syndrome but fed on cow's milk mixture, gives loose, mucous, sometimes green stools. When the dyspeptic infants are treated with an addition of cow's milk or calcium caseinate, the colonic peristalsis and resulting symptoms are not altered (cf. Chapter VI), but an increase of firmness may follow due to an increase in the solid components of the feces, which has been sometimes observed.

In the dyspepsia the stools are often *green.* The green coloring

matter is biliverdin produced from bilirubin by an oxidizing process (by an oxidizing ferment, as Wernstedt demonstrated). Freudenberg and Heller have pointed out that this oxidizing process occurs mainly in an acid environment and at a pH of 5.4 or below. There is probably also an increased amount of bile pigment in the feces, since the colonic resorption deteriorates due to the rapid passage. A varying amount of *mucus* is also existent in the stools of infants with the dyspepsia. It is a known fact that the secretion of mucus increases on parasympathetic irritation, both centrally (cf. among others Boldyreff and Grace, Holman, Wolf and Wolff) and locally by the application of solutions of cholinergic drugs (Jones and White). In the same way as hyperperistalsis and pains, this symptom can also be occasioned by a parasympathetic predominance.

2. Pains

L. Schiff summarizes the knowledge in this field by stating that visceral pain is experienced when the nerve endings in the musculature or submucosa of a hollow viscus are adequately stimulated, the stimulus usually being either stretch or pressure. Sudden distention in the presence of muscular tone or marked contraction involving a broad band of musculature constitutes an adequate stimulus. The intensity of the pain is proportionate to the speed with which the intravisceral tension develops and the height which it attains. Experiments with balloon distention (among others Hertz, Jones, Bingham, Ingelfinger and Smithwich) have demonstrated sensations of pain in different parts of the intestinal tract. Ivy et al. found that a wave of activity in one part of the colon caused propulsion of the bowel contents only if the distal segment accepted the wave and contracted similarly. The frequent failure of the distal segment to do this may cause cramp-like sensations and may explain the symptoms of an irritable colon. Jones and White have by stimulation of the autonomic nervous system with particular emphasis on parasympathetic over-activity (oral administration of Acetyl-beta-methylcholine and Doryl) obtained the same symptoms on healthy students as in mucous colitis, i. e. hyperemia, thickening of the rectal mucosa, muscle spasm and mucous secretion etc, and in addition symptoms of increased lower bowel activity. The same symptoms are obtained in persons with this disturbance produced by psychic stress. Brun on the other hand has

produced colic-like pains by administration of a weak adrenaline solution in the wall of the colon and considers them to be caused by a dilatation of arteries in the tissue below the anemic area of the mucosa. A gastro-intestinal allergy may also cause pains.

Attacks of pain are an almost obligate phenomenon in *the dyspepsia*. It was possible to establish in the roentgen studies that the infants developed colic attacks at the same moment as a contraction was observed in the sigmoid colon and that the colic disappeared in the same second as the contraction. Sometimes the sigmoid colon, and sometimes also parts of the descending colon, have been so contracted that they were visualized in the roentgen examination as a thin string formed by only a few streaks of the contrast medium. This is in agreement with Ivy's results above. The investigation also demonstrated that the contractions in the sigmoid colon are stages in the propulsive motility of the bowel which could be followed from the duodenum to the rectum. The reason for the pains may be considered as (1) a higher pressure above the contracted part of the bowel, (2) that the actual contraction or cramp as such produces attacks of pain, or (3) a local ischemia caused by the spasm in the bowel and consequent prevention of blood circulation. Numerous observations in my investigation demonstrate that *the attacks of pain come when the sigmoid colon undergoes violent contraction*. The same localization is found in infants with spasmus coli where it is possible to palpate the sigmoid colon like a taut thin string in the same place as the vigorous contraction appears during the attacks of pain in my roentgen examination. In Chapter IV the relation of the pains to mealtimes was shown, as also the time of onset and the way in which they occur particularly in connection with feeding, where sucking comprises a predominant factor. In isolated cases attacks of pain have occurred merely by sucking a teat.

An increased pressure in the rectum also produces sensations of pain, and this symptom and the high rectal pressure associated with the dyspepsia is dealt with in Section C, p. 79.

I conceive of *the attacks of pain as a stage in a hyperactivity in the colon due to a failure of balance in the vegetative system with a parasympathetic predominance*. This may be due to an undeveloped central nervous inhibition or control (cf. Section A). But behind this lies probably also the neurolability of the infants and the fact that the failure of balance is a part of the neurolability and an expression of its vege-

tative phase. This explains the fact that this symptom, as also other symptoms of gastro-intestinal origin, generally disappear within the first three to four months, since the higher centers (in the hypothalamus and cortex) are by then developed so that in the main they control the intestinal function. There still remains, however, a tendency to pains in this region, and the comparatively common occurrence later of such pains of the type of spasmus coli in these infants, particularly under mental strain, has been described in Chapter III : 5, indicating a persistent lability in the vegetative nervous system.

Brennemann describes the symptom colic, which as regards symptomatology, duration, the age and neurolability of the infant etc. corresponds to the pains incurred in so-called dyspepsia in breast-fed infants. He states that it occurs more frequently and more violently in the breast-fed baby, that it manifests itself in intermittent, paroxysmal attacks of sharp, intense pain clearly referable to the intestinal tract, and that the pain is agonizing, not a mere discomfort. The babies are fussy, irritable, uncomfortable, crying, "neuropathic". He also emphasizes two other outstanding factors, that colic rarely occurs in the morning and that its occurrence is almost restricted to the baby in the home. His observation that in the majority of infants the colic is most pronounced in the late afternoon or early evening agrees with my observations, even if the colic often occurs at every meal. But it is often more intensive and of longer duration in the evening. I have interpreted this condition as tiredness in these easily tired neurolabile infants, which would more readily induce contractions in the sigmoid colon, as also many of them later develop pain in the same place as a result of mental strain. In the present investigation there are many examples of infants who, in spite of hospitalization, still have equally troublesome pains as at home, the infant not becoming free of distress until after treatment with Skopyl. But many infants have been improved by hospitalization, mostly as regards their neurolabile symptoms but also their colic, and I consider these improvements to be due to the more peaceful environment (cf. Chapter VI on treatment).

3. Gas

Soveri (1939) investigated the gas conditions in the intestinal tract of infants. He found that more air entered the stomach as a result of slow, phlegmatic sucking than when the same amount of liquid

was consumed in a shorter time. The passage through the small bowel took ½—1½ hours, but if the bowel contained other matter the gas passed through more quickly than the contents. The passage through the empty colon took 1—2 hours. He further pointed out that an act of swallowing was required for air to penetrate into the infant's stomach. Magnusson (1931) found, on inflation of the bowel of adults, a rapid passage of air through the intestinal tract, and obtained flatulence after as short a time as 30 minutes. Soveri came to the same conclusion as Magnusson and Engström (1932) that the swallowed air was largely responsible for meteorism in infants.

Mothers of infants with the dyspepsia complain of them suffering from flatulence and meteorism. Brennemann considers that in the colicky baby an excessive amount of gas accumulates in the intestine, especially fostered by the highly fermentable content of breast-milk. In the present investigation no difference was observed in the roentgenograms of these and the normal infants. Nor are the mothers' statements of gassiness in the dyspeptic infants confirmed by the roentgenograms. The reason for this discrepancy may be the greater sharpness of observation in the mothers who watch anxiously over their children. It is conceivable that the increased rectal pressure in these infants causes the passage of gas in small portions but more noticeably, whereas in normal cases nothing at all is noticeable; or that an explosive defecation is interpreted by the mother as a passage of gas, just as many believe that rumblings and gurglings in the intestine are caused by gas. On examining infants with the dyspepsia one is struck by the fact that they generally have an increased tonus in the abdominal muscles and they have increased pressure in the rectum and possibly in other parts of the intestinal tract. These infants often stop feeding sooner than others on account of irritation and pain, but ordinarily they do not receive a smaller amount of food nor require a longer sucking time. An exchange of gas takes place between the bowel content and the blood through diffusion which continues until equilibrium is reached. The oxygen is largely absorbed while the nitrogen remains relatively unaffected. This equilibrium has probably been reached when the gas arrives in the colon, but if this does not happen there may be poorer diffusion on account of the rapid passage and the large mucous content, even if facilitated by an increased pressure (Schoen). A deterioration of the flow of blood in the intestinal wall which might exercise an un-

favorable effect on the absorption is not to be expected. Nor is it to be expected that there would be an increase of fermentation in the intestinal tract of these infants compared with other breast-fed babies. No difference in the content of carbohydrates in the food has been found (cf. among others Raczynski). Jundell has shown that, under normal conditions, only an insignificant or no bacterial content is found in the small bowel, in addition to which the passage through the colon is more rapid in dyspeptic than in other infants. *I find therefore no corroboration nor grounds for an assumption that infants with the dyspepsia have a higher gas content in their intestines than other infants.*

4. Vomiting

Hatcher and Weiss (1923) found in the vicinity of the vomiting center a center for defecation where, by the application of drugs, they were successful in producing nausea and vomiting and often diarrhea and defecation as well. Alvarez, who maintained that there are different kinds of gradient in the small bowel, including a grading down of force of contraction and tone, says that it is possible that reversal of the intestinal gradient will sometimes cause vomiting. In certain cases he conceives of the possibility that a great rise of tonus in the jejunum, where it receives a large branch from the vagus, may give rise to vomiting. Pearcy and Van Liere obtained on distention of the rectum of the dog salivation, nausea and protracted vomiting. Wolf has reported that nausea is accompanied uniformly by gastric relaxation, hypotonicity and hypomotility. Youmans points out that there is evidence that the vomiting which is elicited by intestinal distention is dependent upon thoracolumbar afferent nerves from the bowel. Czerny and Keller assert that vomiting is a usual phenomenon in infants with neuropathic diathesis.

In the present investigation, apart from the regurgitation usual in infants — which is equally common in the normal group — a tendency to vomiting has manifested itself in *infants with the dyspepsia*. The cause may be the same as that of the other intestinal symptoms, a failure of balance with a parasympathetic predominance due to a labile regulation on the part of the central nervous system. It is possible that the increased rectal pressure in these infants may have the same effect

as the distention of the rectum referred to above. It is in fact not surprising that these neurolabile infants with their hyperactive and hypersensitive gastro-intestinal tract have a greater tendency to vomiting than other infants.

C. Pressure conditions

Alvarez, as long ago as 1913, observed a downward gradation in tonus in the small intestine. This may explain the discovery frequently made that liquids will run long distances through the small bowel without the help of any peristaltic contraction. Youmans states that distention of the intestine causes discomfort in a dog when pressures of 60 to 80 millimeters of mercury are used. He has also demonstrated a gastro-intestinal inhibition from anorectal stimuli, even when occasioned by so mild a stimulant as a catheter. He moreover finds that a distention of any part of the small intestine may elicit immediate inhibition of the entire small intestine, including the site of distention, and that repeated distentions at a given site sensitize the segment so that it becomes easier to elicit the reflex from this site. Youmans found further on examination of the jejunum in the dog that denervated segments were more excitable and responded more violently to distention than the segments which were not denervated. Learmonth and Markowitz found in dogs that the lumbar sympathetic nerve exerts a constant inhibitory effect on the musculature of the distal colon. Its section immediately raised intracolonic pressure and sometimes increased the amplitude of the contractions. Zimmermann (1909) found that a change in the rectal pressure from 2 to 3 mm. mercury could be perceived by the persons he studied, that at 20 mm. mercury there appeared a strong sensation of pressure and need of defecation, and at 60 mm. mercury there was an extremely uncomfortable feeling of fullness and tension and acute need of defecation. When Steggerda and Clarc injected 200 c.c. air in the lower colon of adults, a pressure was usually recorded of 20—25 cm. water, but a relatively unchanged pressure on continued administration of air (up to 1 000—1 200 c.c.). At approximately 600 c.c. rhythmic contractions occurred in the colon and there was an increase of amplitude with an increase in the amount of gas. They found that the increased distention of the colon was not accompanied by any distress. Jones found

on distention of the colon with balloons that the pains were far less acute than in the upper part of the digestive tract. Langworthy and Rosenberg (1939) described a tonic mechanism in the midbrain controlling the smooth muscle of the rectal wall. Ström and Uvnäs (1950) observed contractions of the rectum on hypothalamic stimulation.

In the present investigation the pressure in the rectum was measured with a heart catheter and appurtenant apparatus. Investigations were made (cf. Chapter V) of 16 infants, of which 3 normal. The normal infants, while feeding, exhibited a rectal pressure below 60 cm. water, while those with severe dyspepsia exhibited high pressure, the majority attaining a maximum of above 100 cm. water pressure. In the latter, moreover, there were great changes of pressure and amplitudes of up to or above 100 cm. water, while in the normal infants they did not exceed 25 cm. water. In as far as this high rectal pressure is comparable with the rise in pressure obtained on distention of the rectum it would explain symptoms such as abdominal discomfort, restlessness etc. as described by Alvarez, Donaldsson and others. Hines, Lueth and Ivy distended a toy balloon in the rectum and found that the subjects of their experiments, who were students, suffered brief painful sensations increasing in frequency and severity as time went on. They found that in those with a tendency to constipation the rectum was far less sensitive. But if the students suffered discomfort from the increased pressure — and without fluctuations — it is understandable that the sensitive dyspeptic infants react far more strongly, and that their reactions are of the nature of those described by Alvarez and Donaldsson or even more violent. I imagine the increased rectal pressure to be a consequence of the hyperactivity of the colon.

I have not investigated the pressure in the colon. At the times when a violent persistent reaction occurs in the sigmoid colon, the pressure on the two sides must be independent of one another. Otherwise, in roentgen studies of the dyspeptic babies, one seldom sees such a contraction of the colon as might be imagined as completely separating two areas with large differences in pressure. Possibly the large amplitudes, i.e. the differences between the highest and lowest pressures, which characterize the pressure conditions in the rectum of the dyspeptic infants, may be explained by the fact that two separate cavities now and again make contact with one another. It is, however,

more probable that it is the intestinal wall which contracts more violently in dyspeptic than in normal infants. The high pressure in the rectum and the fluctuations in the intensity of pressure may explain how some of the babies' discomfort comes about.

D. Allergy

Calzada Rodriguez found on investigation of 20 infants with prandial diarrhea that they usually showed signs of exudative diathesis. They often had eczema and dermographism. He states, however, that "anaphilaxia or food sensibility does not play any important part in the aetiopathogeny". J. A. Anderson considers that colic in infants is in some cases due to food allergy.

Gray, Harten and Walzer found that after having sensitized the intestine by intramucosal injections — on experimental subjects with fistula — an acute edema was produced within 5—8 minutes combined with hyperemia and an excessive secretion of mucus when the person was given by mouth the substance he had been sensitized against. These lesions occurred both in the colon and small intestine. Walzer et al. found on intravenous injection of antigen in the monkey that pallor and edema accompanied by hyperperistalsis and spasm occurred within about one minute in the passively, locally sensitized mucous membrane of the bowel. Contraction of the smooth muscle has also been demonstrated by, among others, Deissler and Higgins who sensitized a guinea-pig against protein and, on adding a little of it to the solution in which the later excised gall-bladder was preserved, obtained a violent contraction. On roentgen examination of infants with gastro-intestinal allergy, changes have been observed when the allergen was given with barium by mouth or by rectum. Thus Fries and Zizmor record various disturbances such as gastric retention, increased or decreased motility of the gastro-intestinal tract with spasm or dilatation of the stomach or intestines. The clinical observations of the disorders in connection with allergen enema show how it is rapidly followed by a sensation of discomfort and tenesmus, which was not the case when enema was administered without admixture of allergen. They found in infants with gastro-intestinal allergy, on giving the allergen by mouth, nausea in 43 per cent, vomiting in 23 per cent, abdominal pain in 20 per cent, constitutional reactions in 7 per cent, oral

and pharyngeal symptoms in 7 per cent, and in 27 per cent no symptoms were observed. They mention tenesmus on rectal administration, but neither tenesmus nor diarrhea is referred to on oral administration. Schloss treated for gastro-intestinal allergy with antihistaminics and found, out of 41 patients examined, abdominal pain in 33, a fullness sensation in 29, nausea in 11, vomiting in 4 and diarrhea in 23. She states that vomiting occurred several minutes after ingestion of the barium-allergen meal, but does not mention the time at which diarrhea occurred. It is probable that she would have mentioned it if the interval between ingestion and diarrhea had been short, as short as in the dyspepsia. In the two investigations referred to, abdominal pain appears as an essential symptom. According to Chobot and others gastro-intestinal symptoms seldom appear as the only symptoms of allergy.

Thus in gastro-intestinal allergy frequent, loose stools may follow. But not even in a so severe form of food allergy as cow's milk idiosyncrasy is it found, except possibly on rare occasions, that the intestinal reaction in the form of frequent, loose stools occurs so rapidly after ingestion as in the dyspepsia. There are then practically always other symptoms as well. Vendel found in the four Children's Hospitals in Stockholm during the years 1914—1947 cow's milk idiosyncrasy in 23 cases. Of these cases, 14 reacted with diarrhea, of which 4 "immediately" after exposure. But in none of the infants was diarrhea the only symptom of hypersensitivity. It appears inconceivable that a food allergy in 9, 23 or possibly 30 per cent of all infants (which is the frequency of the dyspepsia at a Child Welfare Center in Stockholm, cf. Chapter III, Section 5) should be brought about by the most satisfactory nutrition available, the mother's milk, but should not so readily be caused by other kinds of milk. In addition, there is in the case material no predominance of allergic manifestations (and no case of gastro-intestinal allergy) in the infants with the dyspepsia in relation to the normal infants, nor in their parents and siblings. The result was the same at the investigation at the Child Welfare Center apart from a comparatively speaking rather greater frequency of allergic or suspected allergic manifestations in parents and siblings of the dyspeptic infants.

My experiments with different types of nutrition and the elimination of various elements in ingestion have resulted in a propulsive colonic motility occasioned by different types of food and even a motility

occasioned purely by sucking. *This proves that the symptoms in the dyspeptic infants cannot be due to an allergy* (at least not otherwise than in exceedingly rare cases). No tests have been made with adrenaline or antihistaminics, nor has an investigation taken place of the infants' blood eosinophily.

E. Neurolability

In Chapter III was established that at least 90 per cent of the infants with the dyspepsia exhibited neurolabile symptoms. These symptoms are already apparent in the Maternity Ward and on arrival in the home. They are often aggravated when the intestinal disorders are most intense, but persist, often in a milder form, on after-examination. Not only the infants, but their parents and siblings as well, commonly exhibit signs of a neurolabile disposition, and the same circumstance is apparent from the investigation at the Child Welfare Center.

Experimental and clinical evidence, says Yaskin, suggests a coordination between vegetative and emotional centers, largely situated in the diencephalon, which have a marked influence upon the endocrine glands, metabolism etc. as well as receiving impulses from and sending them to the old and new brain and the neuraxis. This interaction is apparent in the dyspepsia mentally, emotionally, vegetatively and intestinally. The infants, more sensitive and more easily exhausted than normal infants, come into a circle where the "somatic" symptoms increase the neurolabile, and the neurolabile, via vegetative centers, accentuate the "somatic" symptoms. In Section B 2 of this Chapter the influence of mental strain has been pointed out, particularly on the action of the colon. This is also apparent in the present investigation in the influence of change of environment, an improvement in the symptoms often taking place on changing from a restless to a peaceful environment, and the contrary. The same thing sometimes occurs too in individual cases: a 3½-month-old baby which had for one month been free from the dyspepsia, happened to be lying near a telephone and was so frightened when it rang that the baby screamed uncontrollably for hours, the loose openings and pains returning at the same time. Not until Skopyl was administered after a week did the baby recover.

The syndrome can thus be established as being typically psychosomatic.

It must be a matter of great concern that during this sensitive and important stage in their development the infants become free of their often intensive intestinal disorders. It is impossible to do more than make a guess at the influence on the infant and its future mental development of a strain, sometimes approaching maximum, and of abdominal pain and discomfort lasting for weeks and perhaps months, from which release only comes in short periods, if at all, and followed by screaming, anxiety, diminished rest and sleep. But it appears to me probable that the neurolability of the individual is disagreeably intensified, that he becomes set in an abnormal pattern of reaction and that there is risk of permanent future after-effect, possibly a basis for an adult neurosis and a formation of complicated conditioned reflexes with future psychosomatic gastro-intestinal disorders in consequence.

Against this background the necessity of treatment of such infants is apparent: primarily with an anticholinergic drug, and to implement this treatment an adaptation of the environment. It is undoubtedly of importance that the mother be given the opportunity of continuing breast-feeding with all the advantages derived from contact between her and her child, security, affection for her child etc. On many occasions I have given sedatives in similar circumstances, but have not found the results encouraging.

F. Heredity. Frequency

In the previous Section reference was made to the circumstance that not the dyspeptic infants alone but also their parents and siblings are neurolabile to a greater extent than the normal infants and their relatives. It was moreover established at the Child Welfare Center that, in families where some member of the family had "nervous" gastro-intestinal disorders, the children were to a high degree neurolabile. The percentage was 80 in the case of the latter, while of the other children only 7 per cent were neurolabile, cf. p. 36.

But not only neurolability in the common sense but also various kinds of gastro-intestinal diseases, in which a pronouncedly psychic factor is present, are found in the parents and siblings of the dyspeptic infants to a greater degree than in those of the normal infants (cf. Tables 3, p. 22, and 4, p. 23). The investigation shows that, in 85 cases of 102, also other members of the family than the dyspeptic child have or have

had "nervous" gastro-intestinal disorders. The investigation at the Child Welfare Center shows a similar condition in parents and siblings (cf. Chapter III, Section 5, and Table 9, p. 33). In spite of the short time of observation there is apparent in the case material a sensitivity in the gastro-intestinal tract after the dyspepsia has receded, which manifests itself in a tendency to diarrhea. It is found at the Child Welfare Center that children, who have had the dyspepsia, more often than others develop under mental strain symptoms from the gastro-intestinal tract such as abdominal pain and sometimes nausea or vomiting, diarrhea or obstipation, which are the same as occur in spasmus coli. *This concurrence of neurolability and various forms of "nervous" gastro-intestinal disturbances in the dyspeptic infants and their families point clearly to the existence of constitutional factors.*

The case material comprises 111 infants with the dyspepsia, of which 50 in a severe form. Of the parents of the latter 9 had had the dyspepsia, while the corresponding figure for parents of the remaining infants was 4. As regards severe irritable colon, the corresponding figures are 19 and 5. An examination of siblings of the dyspeptic infants — out of 47 siblings there were 19 of infants with severe dyspepsia — reveals that 10 out of the 19 had had the dyspepsia. There are 9 cases of dyspepsia among the other siblings. The corresponding figures for pains of the spasmus coli type are 9 and 4 respectively. The same applies to neurolability, about 85 per cent of mothers of infants with the dyspepsia in a severe form exhibiting a medium or severe neurolability (against about 70 per cent of mothers of infants with dyspepsia in a mild or medium form). Likewise, as regards siblings, the figures of neurolability are about 70 per cent and 35 per cent respectively. *There is thus a more common concurrence of "nervous" gastro-intestinal disorders and neurolability in the parents and siblings of children with the dyspepsia in a severe form than of those with less pronounced dyspepsia.*

In the present investigation there is a *predominance of boys* among the severe cases, 36 out of 50. This was also the case in the Child Welfare Center, where out of 35 dyspeptic infants 21 were boys.

I have not heard of any frequency investigation corresponding to that described in Chapter III: 5. This investigation at a Child Welfare Center showed that about 30 per cent of the children had had the dyspepsia, of whom 9.2 per cent in a severe form. Admittedly B r a c k e t t

(1950) found that out of 195 infants (at a well-baby clinic, at a hospital, and at his private clinic) there was in 68 cases what he calls "diarrhea in the otherwise healthy infant", while 75 did not have the diarrhea, and of the remainder, 52 infants, the records were dubious. He does not, however, make any distinction from the adaptation in the first weeks of life, so that his material and mine are not strictly comparable.

G. Treatment

The transmittance of impulses from the intrinsic and extrinsic nervous system to the functional organs in the bowel (muscle cells and glands) occurs according to the chemical mediator theory by release of acetylcholine from the cholinergic nerves and of noradrenaline (von Euler, 1946) from the adrenergic nerves. Acetylcholine has in general an excitative action on the bowel, i. e. it increases tone and peristalsis.

The belladonna alkaloids, to which group belongs scopolamine and thus Skopyl, prevent the action of acetylcholine on the cells. By means of this blockade of the action of acetylcholine there occurs, among other things, a decrease of tone and of peristaltic activity in the bowel. On the administration of a large dose of atropine (Adler, Atkinson and Ivy, 1942) there is a decrease both of propulsive and non-propulsive motility of the colon. Gilman states that while atropine in general decreases the tone and peristalsis of the gastrointestinal tract, its effect is much more prominent when spasm or hypermotility is present. Alvarez asserts that in his experience the peristaltic rushes in the small bowel were uninfluenced by atropine.

Grace, Holman, Wolf and Wolff found from studies on the colon of man with colostomy that experiments with a parasympathomimetic agent (Acetyl-beta-methylcholine) produced increased motor activity, hyperemia and alteration of fragility. Their observations proved in general to support the view that cholinergic nerve fibers augment the motor, secretory and vascular function of the colon. On injection of a sympathicomimetic agent (Epinephrine) there was a blanching of the colonic mucosa with a sharp diminution of motor activity 13 minutes after the injection, and this effect persisted for 14 minutes. The lysozyme concentration fell. In experiments with autonomic

blocking agents they observed, on intravenous injection of atropine, a profound decrease in motor activity on the left and right sides of the colon persisting for 32 minutes. This was associated with dilatation and a slight blanching of the colon. The response of the colon to the agents tested apparently depended on whether they reinforced or opposed other forces acting on the colon at the time. Strong impulses acting on the bowel as part of the reaction of the individual to his environment were often powerful enough to outweigh the pharmacologic effects of even large doses of the administered agent.

The colonic changes with hyperperistalsis, increased secretion of mucus, pains and augmentation of pressure in the rectum, all of which are found in *the dyspepsia,* correspond to the effect of an increased activity in the cholinergic nerves. The proper treatment should be to administer a counteractant. The belladonna alkaloids are efficient anticholinergic agents. Atropine, on account of its side-effects and toxicity, is not used as a depressor of intestinal motility for the attainment of maximal efficiency. Skopyl (Methylscopolamine nitrate) is appreciably superior to atropine as an anticholinergic agent. The side-effects may become apparent in the influence of Skopyl in restricting the secretion of saliva, mydriasis may develop, and at times there is in sensitive persons a central excitation which is easily eliminated by small doses of phenobarbital. The toxicity of Skopyl is insignificant in the doses administered in the present investigation. Advantage is gained in the simple administration, drops of the alcoholic solution being given sublingually ¼—½ hour before feeding.

Treatment of the dyspepsia with Skopyl has given excellent results clinically. The infants' gastro-intestinal disorders have generally disappeared immediately and their intestinal activity has become normal (in 54 a rapid effect on stools, remaining 7 improved; in 47 rapid effect on pains, 7 improved, 2 unchanged). The neurolability of the infants was also influenced, their screaming fits stopped or diminished and their sensitivity became less acute. Since the commencement of treatment, the propulsive motility of the colon on feeding, revealed in the roentgen studies earlier, disappeared, and the colon developed a width similar to that found in the normal infants, while previously, even before mealtimes, it appeared as a thin string. The pain attacks recorded earlier in the investigation have not recurred. Rectal pressure has after treatment exhibited values approaching those in the normal infants. The

neurolabile symptoms of the infants have also been affected, which is understandable due to the cessation of pain and of the symptoms of increased rectal pressure such as abdominal discomfort, restlessness etc. — not to mention the breaking of the vicious circle formed by the reciprocal action which takes place between the intestinal and neurolabile complexes.

It often proves, however, that the infants' pronounced symptoms of neurolability are not affected by treatment with Skopyl to the degree that is desirable and possible. A change of environment, i.e. hospitalization, has often brought about a decided improvement in neurolabile symptoms. The more easily affected symptoms are screaming and the inability to sleep, or to sleep quietly, at night, while sensitivity to noise and light may be affected but not so strongly.

No beneficial effect on the gastro-intestinal or neurolabile symptoms has been evident as a result of cow's milk or protein therapy, either clinically (apart from an at times somewhat firmer consistence of the stools) or by roentgen examination. The infants treated have not differed from untreated. There is no call to deal here with cases in which the addition serves to compensate for undernourishment.

Brennemann states, in relation to colic, that an enema may bring immediate relief with free expulsion of flatus, but not always. Since it is known that an irritation or an increase of pressure in the rectum (which moreover decreases again) has an inhibitory effect on the remainder of the intestinal tract (Youmans), it is understandable that a certain amelioration can sometimes be achieved by a simple water enema. I cannot imagine, on the other hand, that a soap suds enema is anything but harmful in a sensitive bowel, which already exhibits such pronounced signs of hyperactivity, any more than the administration of castor oil by mouth. It is conceivable that a certain effect can be achieved by a drainage tube or a rectal tube, thus diminishing the rectal pressure.

The good effect of Skopyl treatment on the intestinal symptoms is a further corroboration of their being caused by a parasympathetic predominance. According to Sollman, atropine relaxes intestinal spasm due to parasympathetic stimulation but is not very potent against other intestinal stimulation. With a view to intestinal and neurolabile symptoms and their reciprocal action, *it is certainly a matter of importance that the so-called dyspepsia in breast-fed infants be treated*

as soon as it is established that the complexus is causing distress. Treatment must continue sufficiently long for normalization of the bowel activity to be permanent after the conclusion of treatment. There is undoubtedly an appreciable risk that, unless treatment is given, the neurolabile predisposition and the symptoms thereof in infants which are lacking in powers of resistance may increase and persist on account of the painful and distressful anomalies of intestinal function. *But at times the infants manifest such severe neurolabile symptoms, even after disappearance of the gastro-intestinal ones, that treatment, possibly by change of environment, is indicated. There is no reason to interrupt breast-feeding, when so good a medicine as Skopyl is available, even if sucking is an important factor in initiating the intestinal disorders.* The close contact with the mother gives a sense of security and has in general a good influence on these infants and their sensitive nervous system, and it must therefore be of great value to them to be fed at their mother's breast sufficiently long.

H. Other problems of the dyspepsia

In the course of the years, many hypotheses have arisen regarding the cause of so-called dyspepsia in breast-fed infants and problems connected with it. A difficult question to explain has been *why it is mainly breast-infants that are afflicted.* My conclusions may be summarized as follows: — 1. Most infants of this age are breast-fed (at least in Sweden). 2.The intestinal symptoms are less easily provoked when food is given by spoon or bottle than by breast-feeding. 3. The mothers are more concerned to give the breast to infants with this syndrome, since they appear to have greater need of their mother and her care than other babies. 4. These infants are often difficult to feed by spoon or bottle and attempts to give additional feeding or to wean early are sometimes unsuccessful. 5. My case material consists of 12 infants on additional feeding and 7 purely on cow's milk mixture, which proves that the complexus also exists in other than breast-fed infants. 6. *The type of food* is of no importance, as appears from the experiments in giving different kinds of nourishment (Chapter IV B). The commencement of propulsive motility of the colon 1½ minutes after ingestion can scarcely be caused by the nature of the food.

M a r f a n maintained that he found this complexus more often in

higher *social classes* than in lower, in town children more often than in country, and in the *first-born* more often than in other members of the family. Though I have not made a systematic investigation of the fathers' professions, living conditions etc, I cannot find any indication in my material that a higher social class is afflicted more than others. Marfan's results may perhaps be explained by the fact that his investigations were made in 1923, when the children of lower class parents did not, as now, receive the same medical attention for so commonplace a complaint. Nowadays the majority of children of all classes, at least in Sweden, receive medical attention at the Child Welfare Centers. There may also be a difference between the conditions in France and Sweden. My investigation does not either indicate that the first-born are more afflicted than other children. The former — being then the only child — later exhibit neurolabile symptoms more commonly than others, but the predominance is here undoubtedly due to environment and not to predisposition.

Lönnerblad (1951) found in ten-year-olds a longer time of transit through the small intestine with a small quantity of contrast medium, while a larger quantity took a shorter time. The case is probably similar during the first months of life, and may to some extent apply on *over- or undernourishment.* It is not to be expected, however, that the amount of food given would exert any particular influence in the case of the dyspepsia, in which the time of transit through the small intestine has proved to be very variable and the colonic peristalsis has proved not to be influenced by the amount of food given. There is no therapeutical reason for giving more frequent meals, since they would be accompanied by a more frequent outbreak of intestinal disorders.

Increase in weight. The colon, as already stated, has the important function of resorbing fluid, and it is this function which is impeded by the rapid passage through the colon and possibly by the rich content of mucus in the intestinal wall. In none of the 111 infants comprising the case material has the loss of fluid been so great that a lowered turgor could be determined. The fact that the loss of fluid did not in any case lead to dehydration should be due to the lessened resorption in the colon being unassociated with disturbances of the small bowel function. In some cases the increase in weight has proceeded slowly but has in the main been satisfactory. In the cases where Marfan,

Finkelstein, Rodriguez and others have obtained a lowering of weight and dystrophia, even at times toxicosis, it should be taken into account that other factors than those concerned with those of so-called dyspepsia in breast-fed infants may have been involved.

Borderline cases. The borderline between the dyspepsia at an age of 3 weeks (cf. Chapter II) and the adaptation of the bowel to its new tasks during the first weeks of life may sometimes differentiate cases which should rightly belong to the dyspepsia group. It is conceivable that the frequent, loose stools, which have been taken as the basis of classification, may at times be less pronounced or altogether absent while the symptom picture is dominated by neurolability and attacks of pain. There is, however, an appreciable parallelism between the degree of neurolabile and of intestinal symptoms in the case material.

Roentgen examination has proved an excellent means of revealing the pathophysiology of the gastro-intestinal tract. As a means of diagnosing the dyspepsia, however, it is only required in special cases.

I. Prognosis

The investigation shows that the infants' gastro-intestinal disorders recede, but that there often remains a tendency to gastro-intestinal affections, especially of a pronouncedly psychosomatic character. This is understandable since the neurolability remains. The tendency to diarrhea may be a risk while the children are small, but in the case material all have come through their gastro-intestinal disturbances without harm.

J. Denomination

In Chapter I are recorded different names under which so-called dyspepsia in breast-fed infants has been classified. As far as I have been able to discover, no distinction has been made earlier from the phenomenon of adaptation which is usual in the first weeks of life. Apart from this, however, the syndrome has mainly been distinguished as a separate entity. The changes in the colon found in this investigation — hyperperistalsis, pains, secretion of mucus and increased rectal pressure — correspond to a parasympathetic predominance. The good results of treatment with anticholinergic drugs also indicate the very probable presence of such a predominance. In view of the fact that practically

all the babies show signs of neurolability, that the remainder of the family are also to a high degree neurolabile, that there are many grounds of comparison with other "nervous" gastro-intestinal diseases, particularly irritable colon, where psychic factors are involved as in the dyspepsia and its subsequent symptom of spasmus coli, and that an environmental treatment is of great value, one may draw the conclusion that, in the dyspepsia, the neurolability is the essential factor and that the gastro-intestinal symptoms are an expression of it. The term, so-called dyspepsia in breast-fed infants, employed in Sweden, is misleading since other than breast-babies suffer from the complaint. The most adequate designation is *Colonic Hyperperistalsis in Neurolabile Infants,* which emphasizes the basic factor of neurolability.

SUMMARY AND CONCLUSIONS

A review of the literature has shown that the etiology of so-called dyspepsia in breast-fed infants is obscure and unestablished, and that various methods of treatment have been employed with varying success.

For the purpose of obtaining a deeper and more positive knowledge of the nature of the syndrome and its adequate treatment, the author has conducted an investigation of 111 dyspeptic and 38 normal infants, and further an investigation of the frequency of the syndrome in a material consisting of 589 children.

1. The author has made a distinction between the so-called dyspepsia in breast-fed infants and the defective adaptation accompanied by frequent, loose stools which may occur during the first weeks of life. The dyspeptic infants have frequent, loose stools which as a rule follow directly upon the meconium discharge and continue beyond 3 weeks of age. A description is given of the characteristic symptom picture which, particularly as regards the neurolability and the occurrence of bowel openings and attacks of pain in relation to feeding, practically always distinguishes the syndrome from other disturbances accompanied by loose stools.
2. More than half of the infants have attacks of pain even after normalization of the stools, and the author considers that the majority of cases of so-called three months' colic are attributable to the syndrome described. After the disappearance of the dyspepsia the children still have a tendency to diarrhea — occurring in about 40 per cent of those after-examined — and pains of the spasmus coli type.
3. The frequency investigation at a Child Welfare Center in Stockholm shows that, out of 589 children, about 30 per cent had had the dyspepsia, 7½ per cent in a mild form, 14 per cent medium and 9.2 per cent severe.
4. The parents and siblings, to a higher degree than the families of the normal infants, apart from having suffered from the dyspepsia in infancy, exhibit gastro-intestinal disorders, often of a psychosomatic character, including particularly pains of the spasmus coli

type in childhood and "irritable" colon as adults. They also exhibit a higher degree of neurolabile disposition.

5. By means of roentgen examination the author has been able to establish a distinct difference between the condition of the colon of the dyspeptic and of the normal infants. Thus in the former, roentgen examination reveals a resistance to contrast enema with an often convulsively contracted colon and further a propulsive motility of the colon on feeding. This propulsive motility of the colon is a continuation of a peristaltic movement starting in the duodenum about ¼ minute after the commencement of feeding, reaching the cecum at about 1½ minutes. It is perceived as a contraction of the sigmoid colon and sometimes of the descending colon also, accompanied by an attack of pain, and terminates in defecation about 6 to 7 minutes after the commencement of feeding. This propulsive motility of the colon gives a rapid passage through the colon. In cases observed, complete passage and emptying took 4.35—8 minutes from the commencement of feeding. This rapid passage through the colon and consequent deterioration in fluid resorption causes looseness of the feces.
6. Roentgen examination of the ventricle, duodenum and small intestine has not revealed any difference in relation to the normal infants. The time of transit from the contrast medium being administered until it reached the colon has in both normal and dyspeptic infants varied in round figures between 2 and 7 hours.
7. It was possible to induce the propulsive motility of the colon by giving either breast-milk or cow's milk mixtures by different means, and on some occasions by the mere act of sucking. This proves that so-called dyspepsia in breast-fed infants is independent of the nature of the food. Intimate contact with the mother without sucking produced no motility of the colon.
8. The rectal pressure and its fluctuations were measured in 16 infants, of which 3 normal. The normal infants exhibited during feeding a rectal pressure of up to 60 cm water with small fluctuations in pressure not exceeding 25 cm water. Children who had had the dyspepsia deviated from the normal infants in the greater fluctuations in pressure. Infants with dyspepsia in a severe form exhibited during feeding a high pressure — over 100 cm water in the majority of cases — and great fluctuations in pressure. The

light and medium cases have exhibited lower values and smaller differences in pressure than the severe cases.

9. Treatment with an anticholinergic drug, Methylscopolamine nitrate, (Skopyl, Pharmacia), produced a rapid effect on the loose stools and attacks of pain, and moreover had a good influence on the infants' neurolabile symptoms. After treatment with Skopyl the colon showed a roentgen picture in complete agreement with that of the normal infants.
10. Change of environment has exercised little influence on the gastro-intestinal symptoms, but often favorably affected other neurolabile symptoms.
11. Cow's milk mixture or protein have had no effect on gastro-intestinal or neurolabile symptoms.
12. Allergic manifestations are not more common in these infants than in normal. Allergy has not proved to be the cause of the syndrome.
13. The author finds that the intestinal symptoms — hyperperistalsis of the colon including relaxation of the ileocecal sphincter, attacks of pain, increased rectal pressure and increased mucous content in the feces — are explained by a parasympathetic predominance, which is further supported by the good results of treatment with an anticholinergic preparation. The author concludes that this failure of vegetative balance with a parasympathetic predominance forms a part of the infants' neurolability. Both the neurolabile and gastro-intestinal symptoms are in a high degree dependent on constitutional factors. The proper denomination of the syndrome discussed in this paper should, in the author's opinion, be *Colonic Hyperperistalsis in Neurolabile Infants.*

RÉSUMÉ ET CONCLUSIONS

L'examen des études consacrées au présent sujet a montré que la cause de ce qu'on appelle "la diarrhée prandiale des nourrissons au sein" est obscure et inexpliquée, et que diverses méthodes de traitement ont été appliquées avec des succès variables.

En vue d'obtenir une connaissance plus profonde et plus sûre de la nature de ce syndrome et de son traitement adéquat, l'auteur a, d'une part, examiné 111 enfants affectés de troubles dyspeptiques et 38 enfants normaux; il a, d'autre part, fait une enquête de fréquence au sujet de ce syndrome, enquête basée sur 589 cas.

1. Pour différencier ce qu'on appelle "la diarrhée prandiale des nourrissons au sein" des selles molles et fréquentes, causées par une adaptation défectueuse, qui peuvent survenir pendant les premières semaines de la vie, l'auteur l'a décrite comme suit: Les enfants affectés de troubles dyspeptiques ont des selles fréquentes et molles qui, en règle générale, suivent immédiatement les évacuations méconiales et persistent au delà de la troisième semaine de vie. Il en a décrit les symptomes caractéristiques, en particulier la névrolabilité et le rapport des selles et des accès de douleur avec les repas, ce qui presque toujours définit le syndrome par opposition aux autres malaises accompagnés de selles molles.
2. Plus de la moitié des enfants éprouvent des accès de douleur même après que les évacuations ont été normalisées, et l'auteur pense que la plupart des cas de ce qu'on appelle "Three months' colic" appartiennent au syndrome décrit. Lorsque la dyspepsie a cessé, les enfants souvent gardent une propension à la diarrhée dans environ 40 % des cas examinés, et des douleurs du type "spasmus coli".
3. Des recherches de fréquence dans une dispensère pour enfants à Stockholm montrent que sur 589 enfants, 30 % environ ont eu ces diarrhées dites prandiales, 7½ % d'une manière bénigne, 14 % d'une manière modérée et 9,2 % à un haut degré.
4. Les parents et enfants ont, à un plus haut degré que la famille d'enfants normaux, des embarras gastro-intestinaux de nature

souvent psychosomatique, en plus des troubles dyspeptiques, en particulier des douleurs coliques du type spasmodique durant l'enfance, et, à l'âge adulte, un colon irritable. Ils montrent même à un plus haut degré une disposition névrolabile.

5. Par l'examen radiologique, l'auteur a pu établir une nette différence dans l'état du colon chez ces enfants et chez les enfants normaux. L'examen radiologique décèle chez les enfants affectés de troubles dyspeptiques, d'une part une résistance au lavement baryté avec un colon souvent complètement fermé, d'autre part une motilité propulsive du colon au moment où l'enfant absorbe la nourriture. Cette motilité propulsive du colon est la suite d'un mouvement qui a son origine dans le duodenum ¼ de minute environ après le début de l'alimentation, qui atteint le caecum en une minute et demie environ et qui se révèle comme une contraction du colon sigmoideum ou parfois même du colon descendens accompagnée d'un accès de douleur et suivie d'une défécation 6 ou 7 minutes environ après le début du repas. Cette motilité propulsive du colon occasionne un passage rapide par le colon. Le passage complet et l'évacuation ont pris dans les cas observés de 4,35 à 8 minutes après le début du repas. Ce passage rapide par le colon et la liquidité accrue qui en résulte sont causes du caractère mou des selles.
6. L'examen radiologique de l'estomac, du duodenum et de l'intestin grêle n'a pas révélé de différence avec ceux des enfants normaux. Le temps de passage depuis l'instant où l'enfant a commencé de prendre le repas baryté jusqu'au moment où celui-ci a atteint le colon, a varié, tant chez les enfants normaux que chez les dyspeptiques, entre 2 et 7 heures.
7. La motilité propulsive du colon a pu être engendrée par l'absorption, sous des formes différentes, soit de lait tiré du sein, soit de lait de vache, et même, dans quelques cas, à la suite d'une simple succion. Ceci prouve que ce qu'on appelle "la diarrhée prandiale des nourrissons" ne dépend pas de la nature de l'aliment. Un contact intime avec la mère, sans succion, n'a provoqué aucune motilité du colon.
8. La pression dans le rectum et ses variations ont été mesurées chez 16 enfants dont 3 normaux. Les enfants normaux ont montré au cours de l'alimentation une pression rectale allant jusqu'à 60 centi-

mètres d'eau, et cela, avec de petites variations qui ne dépassèrent pas 25 cm. d'eau. Les enfants qui ont eu des troubles dyspeptiques se sont distingués des enfants normaux par des variations de pression plus accentuées. Les enfants affectés de ces troubles à un haut degré, ont montré pendant l'absorption de la nourriture, d'une part une pression élevée, la plupart avec un maximum de plus de 100 cm. d'eau, d'autre part de fortes variations dans la pression. Les cas bénins et moyens ont atteint un niveau moins élevé et ont eu des différences de pression moindres que les cas plus graves.

9. Le traitement avec une préparation anticholinergique, méthylscopolamine nitrate (Skopyl, Pharmacia) a exercé une action rapide sur les selles molles et les accès de douleur, et souvent même a eu une bonne influence sur les symptomes névrolabiles de l'enfant. A l'examen radiologique, le colon offre, après le traitement de Skopyl, un aspect qui s'accorde tout-à-fait avec celui des enfants normaux.
10. Le changement de milieu a eu peu d'influence sur les symptomes gastro-intestinaux mais souvent un heureux effet pour les autres symptomes névrolabiles.
11. Le lait de vache ou la préparation de proteine n'a pas modifié les symptomes gastro-intestinaux ou névrolabiles.
12. Les manifestations allergiques ne sont pas plus fréquentes chez ces enfants que chez les enfants normaux. L'allergie ne s'est pas révélée comme la cause du syndrome.
13. L'auteur trouve que les symptomes intestinaux : l'hyperpéristaltisme du colon, le relâchement du sphinctère iléocécal, les accès de douleur, la pression accrue dans le rectum et l'accentuation de la mucosité des selles s'expliquent par une prédominance du parasympathique, ce qui fut confirmé par les bons résultats du traitement basé sur la préparation anticholinergique. L'auteur conclut que cet équilibre végétatif défectueux, accompagné de la prédominance du parasympathique, constitue une partie de la névrolabilité de l'enfant. Les symptomes tant névrolabiles que gastro-intestinaux dépendent, dans une large mesure, de facteurs constitutionnels. Le nom adéquat du syndrome ici examiné doit être, selon l'auteur, *hyperpéristaltisme du colon chez les nourrissons névrolabiles.*

ZUSAMMENFASSUNG UND FOLGERUNGEN

Die Durchsicht der Literatur hat gezeigt, dass die Ursache der sog. Brustmilchdyspepsie des Kindes unklar ist und dass der Erfolg der verschiedenen Behandlingsmethoden ein variierender ist.

Um zu einer tieferen und sicheren Kenntnis der Natur dieses Syndroms zu kommen, hat der Verfasser teils 111 dyspeptische Kinder und 38 gesunde Kinder untersucht, teils hat er eine Frequenzuntersuchung dieses Syndroms mit einem Material von 589 Kindern durchgeführt.

1. Der Verfasser unterscheidet die sog. Brustmilchdyspepsie des Kindes von der mangelhaften Anpassung mit häufigen dünnen Stühlen, welche in den ersten Wochen auftreten kann: die dyspeptischen Kinder haben häufige und dünne Stühle, welche in der Regel direkt nach dem Mekoniumabgang folgen und bis über das Alter von drei Wochen andauern. Das charakteristische Syndrombild, besonders die Neurolabilität und das gleichzeitige Auftreten der Stühle und den Schmerzanfällen mit den Mahlzeiten, welche so gut wie immer das Syndrom gegen andere Störungen mit dünnen Stühlen abgrenzt, wird beschrieben.
2. Mehr als die Hälfte der Kinder haben auch nach der Normalisierung der Stühle Schmerzanfälle, und der Verfasser ist der Ansicht, dass die sog. „three months colic" zu dem beschriebenen Syndrom gehört. Nachdem „die Dyspepsie" aufgehört hat, behalten die Kinder öfters eine Neigung zu Diarrhoe — bei ca. 40 % der nachuntersuchten Kinder — und zu Schmerzen vom colic-spastischen Typ.
3. Eine Frequenzuntersuchung in einer Kinderberatungsstelle in Stockholm zeigt, dass von 589 Kindern ca. 30 % „die Dyspepsie" gehabt hatten, 7½ % von leichterer Art, 14 % mässig schwerer und 9,2 % eine schwere.
4. Die Eltern und Geschwister haben in einem höheren Grad als die Anverwandten normaler Kinder gastrointestinale Beschwerden, oft psychischer Natur, über „die Dyspepsie" hinaus, besonders Schmerzen vom colic-spastischen Typ im Kindesalter und ein

„irritable" Colon als Erwachsene. Sie zeigen auch in einem höheren Grad eine neurolabile Anlage.

5. Der Verfasser hat durch Röntgenuntersuchung eine distinkte Verschiedenheit in den Colonverhältnissen dieser Kinder gegenüber denen normaler Kinder feststellen können. Bei der Röntgenuntersuchung zeigt sich so teils ein Widerstand gegen den Kontrasteinlauf mit einer häufigen krampfartigen Kontraktion des Colons, teils eine propulsive Colonmotilität beim Füttern. Diese propulsive Colonmotilität ist eine Fortsetzung auf eine peristaltische Bewegung, welche vom Duodenum ungefähr ¼ Minute nach dem Beginn der Mahlzeit ausgeht, das Coecum nach ungefähr 1½ Minute erreicht, sich als eine Zusammenziehung des Colon sigmoideum und manchmal auch des Colon descendens mit darauffolgender Schmerzattacke zu erkennen gibt und mit einem Stuhlabgang ungefähr 6—7 Minuten nach Beginn der Mahlzeit schliesst. Durch diese propulsive Colonmotilität kommt eine schnelle Colonpassage zustande: vollständige Passage und Entleerung dauerten in den beobachteten Fällen 4,35—8 Minuten vom Beginn der Nahrungsaufname. Diese schnelle Colonpassage und die darauffolgende verschlechterte Flüssigkeitsresorption verursachen die dünne Konsistenz des Faeces.
6. Bei der Röntgenuntersuchung des Magens, Duodenums und Dünndarms konnte kein Unterschied der Verhältnisse gegenüber normalen Kindern festgestellt werden. Die Durchgangszeit von dem Augenblick, da das Kind Kontrast einzunehmen begann, bis zu dem Zeitpunkt, da dieser das Colon erreicht hat, variierte sowohl bei normalen als auch bei dyspeptischen Kindern zwischen rund 2 und 7 Stunden.
7. Die propulsive Colonmotilität konnte durch Darreichung verschiedener Art sowohl von Brustmilch als auch Kuhmilchmischungen ausgelöst werden, aber in einigen Fällen auch durch einfaches Saugen. Das zeigt, dass die sog. Brustmilchdyspepsie des Kindes nicht von der Art der Nahrung abhängig ist. Intimer Kontakt mit der Mutter ohne Saugen löste keine Colonmotilität aus.
8. Der Rectaldruck und dessen Schwankungen wurde bei 16 Kindern, darunter 3 normale, gemessen. Die normalen Kinder zeigten unter der Nahrungsaufnahme einen Rectaldruck um 60 cm Wasserdruck und geringe Druckschwankungen, die nicht 25 cm Wasserdruck

überstiegen. Kinder, welche „die Dyspepsie" gehabt haben, unterscheiden sich von den normalen durch grössere Druckschwankungen. Kinder mit hochgradiger Brustmilchdyspepsie zeigten bei der Nahrungsaufnahme teils einen hohen Druck, die meisten mit einem Maximum über 100 cm Wasserdruck, teils grosse Druckschwankungen. Die leichten und mässig schweren Fälle haben niedrigere Werte und weniger Druckdifferenzen gezeigt als die schweren.

9. Die Behandlung mit einem anticholinergischen Präparat, Methylscopolaminnitrat (Skopyl, Pharmacia), hat einen raschen Effekt auf die dünnen Stühle und auf die Schmerzanfälle gehabt und oft auch einen guten Einfluss auf die übrigen neurolabilen Symptome der Kinder. Bei der Röntgenuntersuchung nach der Behandlung mit Skopyl, zeigt das Colon ein vollständig mit dem normaler Kinder übereinstimmendes Bild.
10. Milieuwechsel hat einen geringen Einfluss auf die gastrointestinalen Symptome gehabt, aber oft einen guten auf die neurolabilen Symptome.
11. Kuhmilchmischungen oder Eiweisspräparate haben die gastrointestinalen oder die neurolabilen Symptome nicht beeinflusst.
12. Allergische Manifestationen kommen bei diesen Kindern nicht öfter als bei normalen Kindern vor. Die Allergie hat sich nicht als die Ursache dieses Syndroms erwiesen.
13. Der Verfasser findet, dass die Darmsymptome: Colonhyperperistaltik zusammen mit Relaxation des Ileocoecalsphinkters, Schmerzanfälle, erhöhter Rectaldruck und grösserer Schleimgehalt in den Faeces durch ein parasympatisches Uebergewicht erklärt werden, was sichtlich durch die guten Behandlungsresultate mit anticholinergischen Präparaten gestützt wird. Der Verfasser kommt zu der Ansicht, dass dieser Mangel an vegetativem Gleichgewicht mit einem parasympatischen Uebergewicht teilweise aus der Neurolabilität des Kindes hervorgeht. Sowohl die neurolabilen als auch die gastrointestinalen Symptome beruhen in hohem Masse auf konstitutionellen Faktoren. Der entsprechende Name für das hier besprochene Syndrom sollte, nach der Ansicht des Verfassers, *Colonhyperperistaltik bei neurolabilen Säuglingen* sein.

SUMARIO Y CONCLUSIONES

Una revisión de la bibliografía ha mostrado que la llamada dispepsia de leche materna no está ni precisa ni clara y que han sido aplicados varios tratamientos con éxitos distintos.

Para llegar a un conocimiento más exacto y seguro de la naturaleza de este síndrome y a un tratamiento adecuado, el autor ha explorado en parte 111 niños afectados de dispepsia y 38 niños sanos y en parte ha hecho un examen de la frecuencia con que este síndrome aparece en un material de 589 niños.

1. El autor ha delimitado la llamada dispepsia de leche materna de la adaptación defectuosa con defecaciones frecuentes y disueltas que pueden aparecer durante las primeras semanas: los niños dispépticos tienen defecaciones frecuentes y claras que generalmente se suceden directamente a la eliminación de meconio y permanecen hasta más allá de las tres semanas de edad. Se describe el cuadro sintomático característico, especialmente la neurolabilidad y la relación de las defecaciones y los ataques de dolor con la ingestión de alimento que casi siempre separa el síndrome de otros trastornos con eliminación de heces disueltas.
2. Más de la mitad de los niños tiene ataques de dolor incluso después de haberse normalizado las heces y el autor cree que la mayoría de los casos de la llamada "three months colic" pertenece al síndrome descrito. Después de haber desaparecido "la dispepsia" los niños conservan amenudo una tendencia a la diarrea — en aproximadamente un 40 % de los niños controlados — y a dolores de un tipo de cólico espástico.
3. Un examen de la frecuencia en un dispensario de control infantil en Estocolmo ha mostrado que de 589 niños aproximadamente un 30 % ha sufrido "la dispepsia", 7½ % de grado liviano, 14 % de grado medio y un 9,2 % grave.
4. Los padres y hermanos tienen en grado mayor que los parientes de niños normales trastornos gastrointestinales, amenudo de naturaleza psicosomática, más allá de "la dispepsia", especialmente dolores de tipo cólico espástico durante su infancia y de colon "irritable"

como adultos. Muestran además una marcada predisposición a la neurolabilidad.

5. El autor ha podido comprobar mediante la exploración radiológica una diferencia en las condiciones del colon de estos niños comparado con las de niños normales. A la exploración radiológica se demuestra esta diferencia en los niños dispépticos en parte por una resistencia al enema de contraste con amenudo cierre espasmódico del colon, en parte por una motilidad propulsiva del colon a la ingestión. Esta motilidad propulsiva del colon es una continuación de un movimiento peristáltico que parte del duodeno, aproximadamente ¼ de minuto después del comienzo de la ingestión, aparece en el ceco después de 1 minuto y medio y se da a conocer en una contracción del colon sigmoideo y a veces también del colon descendente con un ataque sucesivo de dolor, terminando con una expulsión de heces aproximadamente de 6 a 7 minutos después del comienzo de la ingestión. A causa de esta motilidad propulsiva del colon aparece un pasaje del colon rápido: el pasaje completo y vaciamiento ha durado en los casos observados de 4,35 a 8 minutos desde el comienzo de la ingestión. Este pasaje rápido del colon y la mala resorción de líquido consecuente, son la causa de la consistencia disuelta de las heces.
6. A la exploración radiológica del estómago, del duodeno y del intestino delgado no se ha encontrado ninguna diferencia en las condiciones de aquellas existentes en niños normales. El tiempo de pasaje desde que el niño ha empezado ha ingenir el contraste hasta que éste ha llegado al colon, ha durado tanto en niños normales como en los afectos de dispepsia en una variación global de 2 a 7 horas.
7. La motilidad propulsiva del colon se ha podido provocar a través de la ingestión, de diferentes modos, tanto de leche materna como de mezclas de leche de vaca, pero en algunos casos también con solo la succión. Esto demuestra que la llamada dispepsia de leche materna no es causada por la clase de alimento. Contacto íntimo con la madre sin succión no provocó ninguna motilidad del colon.
8. Se ha medido la presión rectal y sus variaciones en 16 niños, entre ellos 3 niños normales. Los niños normales mostraton durante la ingestión de alimento una presión rectal alrededor de 60 cm de agua con oscilaciones pequeñas que no pasaron de 25 cm de agua.

Niños que han sufrido "la dispepsia" se diferencian de los niños normales por oscilaciones mayores. Niños con dispepsia de leche materna grave mostraron durante la ingestión de alimento en parte una presión alta, la mayoría con un máximo de más de 100 cm de agua, en parte grandes oscilaciones. Los casos livianos y medios han mostrado valores menores o menos oscilaciones que los graves.

9. Eltratamiento con un producto anticolinérgico, nitrato de metil-escopolamina (Skopyl, Pharmacia), ha tenido un efecto rápido sobre las heces claras y los ataques de dolor y además ha tenido también amenudo una buena influencia sobre los demás síntomas neurolábiles de los niños. A la exploración radiológica después del tratamiento con Skopyl, el colon muestra un cuadro que coincide con el de los niños normales.
10. El cambio de ambiente ha tenido muy poca influencia sobre los síntomes gastrointestinales, pero amenudo una de buena sobre los demás síntomas neurolábiles.
11. Ni las mezclas de leche de vaca ni los preparados de albúmina han variado los síntomas gastrointestinales o los síntomas neurolábilis.
12. Las manifestaciones alérgicas no son más frecuentes en estos niños que en niños normales. La alergia no ha demostrado ser la causa del síndrome.
13. El autor encuentra que los síntomas intestinales: peristaltismo del colon con inclusive relajamiento del esfínter ileocecal, ataques de dolor, presión rectal aumentada y aumento del contenida mucoso en las heces, se explican por un predominio del parasimpático, lo que se confirma francamente por el buen resultado obtenido con el preparado anticolinérgico. El autor ha llegado a la conclusión que la falta de equilibrio vegetativo con un predominio del parasimpático proviene, en parte, de la neurolabilidad del niño. Tanto los síntomas neurolábiles como los gastrointestinales tienen en gran parte su causa en los factores constitutionales. La denominación adecuada para el síndrome que aqui se discute tendría que ser, según la opinión del autor, *Hiperperistaltismo del colon en niños neurolábiles.*

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PLATES

a

b

c

So-called dyspepsia in breast-fed infants, severe form.

Passage through small intestine.

23 mins. after contrast meal. Before breast-feeding. — b. 25 mins. after contrast meal. 2 mins. after commencement breast-feeding. — c. 33 mins. after contrast meal. Breast-feeding completed.

Plate 2

a

b

Normal infant. Contrast enema.

a. Before breast-feeding. — b. After breast-feeding.

a

b

c

d

So-called dyspepsia in breast-fed infants, severe form. Contrast enema.
a. Before breast-feeding. — b. After breast-feeding. — c. After Skopyl treatment. Before breast-feeding. — d. After Skopyl treatment. After breast-feeding.

Plate 4

a

b

So-called dyspepsia in breast-fed infants, medium form.
a. Before breast-feeding. — b. After breast-feeding.

a

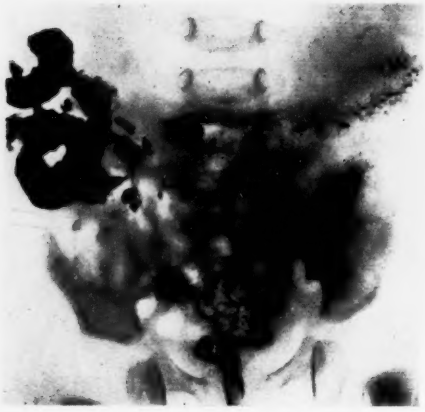
b

So-called dyspepsia in breast-fed infants, mild form.
a. Before breast-feeding. — b. After breast-feeding.

Plate 6

a

b



c

So-called dyspepsia in breast-fed infants, severe form.

a. Before feeding. — b. After feeding by tube. — c. After subsequent breast-feeding.

a

b

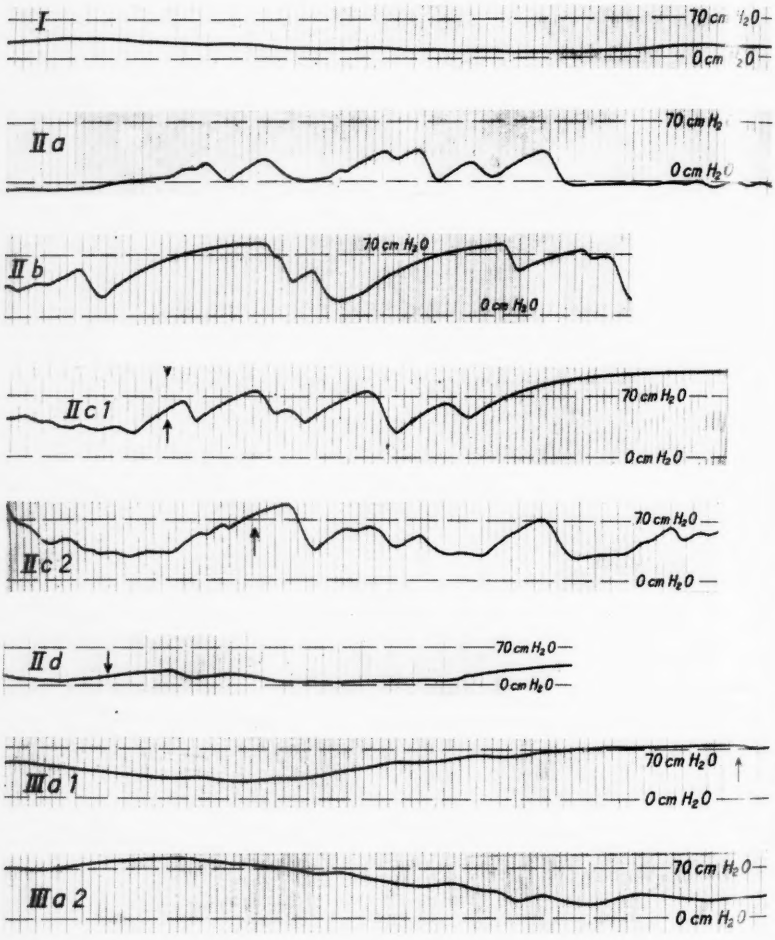
c

d

So-called dyspepsia in breast-fed infants, severe form.

a. Treated with protein. Before breast-feeding. — b. Treated with protein. After breast-feeding. — c. Treated with Skopyl. Before breast-feeding. — d. Treated with Skopyl. After breast-feeding.

Plate 8



Pressure measurement in the rectum. (The time interval between the vertical lines is 1/10 second.)

No. I. Normal case. During feeding.

No. II. Case of severe dyspepsia.
a. During breast-feeding. — b. After breast-feeding.
c 1 and c 2. Breast-feeding. Defecation. Commencement indicated by arrow. — d. After treatment with Skopyl. Normalized. Breast-feeding. Arrow indicates time when slight defecation occurred.

No. III. Case of severe dyspepsia.
a 1. Breast-feeding. The arrow indicates defecation. — a 2. Continuation of the curve. Breast-feeding continues.

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EFFECT OF VITAMIN D ON THE METABOLISM OF RADIOCALCIUM IN RACHITIC RATS

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CHAPTER I

Introduction

Despite extensive investigation by a number of workers in this field, our knowledge of the mode and site of action of vitamin D on healing rickets is still incomplete. Differences in the opinions on this question are to a certain extent ascribable to differences in the experimental conditions used by the investigators. Rickets in rat is usually produced by a cereal, vitamin D deficient diet, in which not only the absolute amounts of calcium and phosphorus, but also their proportion to each other are important. Rickets may differ metabolically with the type of diet used, and then the response to vitamin D may differ accordingly (see REED, STRUCK & STECK, 1939). This is particularly the case when the diet is severely unbalanced with regard to calcium and phosphorus (cf. DAY & MCCOLLUM, 1939, SCHNEIDER & STEENBOCK, 1939), and under these conditions vitamin D deficiency is not always necessary to disturb calcification (COLEMAN, BECKS, KOHL & COPP, 1950). Much confusion in the discussion of rickets seems to be due to the lack of a clear-cut definition of this term. Most authors seem, however, to agree in that rickets is a condition of vitamin D deficiency readily recognized by a decrease in the inorganic phosphorus in the serum and by certain skeletal changes. These consist mainly of a continued formation of matrix (cartilage, osteoid tissue) with a failure of it to calcify (see WOLBACH & BESSEY, 1942). In the experimental investigation of rickets then, the most important thing is to carry out the experiments under conditions that will permit the administration of vitamin D to control the disturbances mentioned above. Rickets of this type can be produced by feeding rats on a diet deficient in

vitamin D with a high Ca:P ratio, in which the absolute value of P is not too low.

Like several other factors controlling calcification, vitamin D may exert a direct or an indirect effect in one or more of the following ways (cf. LOGAN, 1940)

a) By varying the net absorption of calcium and phosphorus from the intestinal tract.

b) By regulating the urinary excretion of these constituents.

c) By altering the rate of deposition and dissolution of inorganic and organic solids in the skeleton.

The absorption of calcium and phosphorus from the digestive tract has been studied by several investigators. In experiments on rachitic rats NICOLAYSEN (1937 A, B) found medication with vitamin D to promote the absorption of calcium from the intestines and thereby indirectly to increase the absorption of phosphorus. Later observations in man by ALBRIGHT & SULCOWITCH (1938) confirmed this finding. These authors, however, studied the net absorption, i. e. the difference between the absorption from, and the excretion into, the digestive tract. The introduction of the tracer technique, made it possible to study each of these processes separately. Using radiocalcium GREENBERG (1945) and HARRISON & HARRISON (1951) found vitamin D to promote the absorption of calcium from the intestinal tract in rachitic rats. Phosphate tends to be absorbed to a greater extent than calcium, even in normal rats (see LOGAN, 1940), and, in the absence of calcium in the intestine, vitamin D seems to have no effect on the absorption of phosphorus. DOLS & JANSEN (1937) found no influence of vitamin D on the absorption of P^{32} from the intestine, while COHN & GREENBERG (1939) claimed a slight effect.

Even though there is agreement about a promoting effect of vitamin D on the absorption of calcium, our knowledge of this mechanism is still far from complete. The absorption of calcium does not seem to differ demonstrably from that of substances wich pass through the wall of the bowel by means of simple diffusion, the rate of which can be increased

by the administration of vitamin D (NICOLAYSEN, 1937 C). Evidence has also been presented that the absorption of calcium occurs in the upper intestinal tract (see REED *et al*, 1939, STEARNS, 1950). Later, however, HARRISON & HARRISON (1951) claimed that vitamin D exerts its effect only under conditions in which the calcium of the intestinal content is poorly soluble, i. e. in the distal part of the small intestine and in the large intestine. But it is obvious that these investigators did not work under conditions permitting vitamin D to exert optimal action. As their series were also small, their observations can hardly be regarded as evidence strong enough to disprove earlier opinions on this point, which therefore calls for further research. Whether the favourable effect of vitamin D on the absorption is exerted primarily on the intestinal mucosa or on the skeleton is not known either. NICOLAYSEN (1943) suggested that the immature skeleton — possibly by the production of a hormone — controls the absorption of calcium. Further, it is not yet known in what state, or how, calcium crosses the intestinal membranes. It is possible that some phosphorylation process in the intestinal mucosa necessary for the absorption of calcium is stimulated by vitamin D. ZETTERSTRÖM (1951) observed in *in vitro* experiments that phosphorylated vitamin D activates alkaline phosphatase from the intestine. Investigation of this point *in vivo* was difficult earlier, because of the unavailability of a satisfactory method for measuring the effect of the vitamin on the absorption from the digestive tract. We also lack relevant knowledge of such fundamental factors as optimal dose and optimal time of action of vitamin D.

In rats, if the phosphate intake is below the requirement and the calcium intake greatly in excess, the calcium is largely excreted with the urine (see LOGAN, 1940). HARRISON & HARRISON (1941) observed that medication with vitamin D increases tubular re-absorption of phosphate, probably by a direct effect upon renal tubular cells. However, ALBRIGHT & REIFENSTEIN (1948) interpret this effect as secondary to a decreased activity of the parathyroids ascribable to increased serum calcium. In the literature there seems to be no reports on a direct effect

of vitamin D on the excretion of calcium by the kidneys. In the initial stage of osteomalacia in man, calcium has been reported to diminish and finally disappear from the urine before the total serum calcium concentration falls appreciably (CHU, LIU, YU, CHENG, & CHAO, 1940).

The uptake of radiocalcium in rachitic bones has been studied by GREENBERG (1945), who supposed a direct effect of vitamin D on the mineralisation of bone salts. In his experiments, however, he did not study and consider the changes of the inorganic phosphorus in the serum following medication with vitamin D. The first to demonstrate a quantitative relation between the concentration of Ca and P of serum and calcification were HOWLAND & KRAMER (1921). In a later paper KRAMER, SHEAR & SIEGEL (1931 A) reported that an increase in the Ca $\times$ P product regularly precedes the appearance of new calcification in healing rickets. HESS, WEINSTOCK, RIVKIND & GROSS (1930) claimed that when irradiated ergosterol was given in adequate amounts to rachitic rats, the phosphate concentration of the blood was raised to normal level in spite of the fact that no antirachitic or calcifying action was demonstrable in the epiphysis of the long bones. Their views were, however, severely criticised and disproved by KRAMER *et al.* (1931 B). The importance of changes in the chemical composition of the blood was outshadowed by the attention given to local processes in the bone tissue, particularly to the importance of ester phosphate and alkaline phosphatase in the calcification mechanism, in which one of the main actions was to produce a local increase in the concentration of inorganic phosphorus at the site of calcification (see ROBISON & ROSENHEIM, 1934). ZETTERSTRÖM (1951) observed in *in vitro* experiments that phosphorylated vitamin D stimulates the activity of alkaline phosphatase from bone tissue. COHN & GREENBERG (1939) found vitamin D to raise the inorganic phosphorus uptake in bone by 25 to 50 per cent in rachitic rats, and that the rate of phosphorus uptake by the organic fraction of bone is more rapid than that of muscle. From this they conclude that vitamin D acts to aid the conversion of organic

to inorganic phosphorus in bone. However, in their material the increase in the ratio P^{32}/P^{31} for inorganic + organic fraction of bone does not seem to be larger than the increase for solely the inorganic fraction after the administration of vitamin D, so that the abovementioned conclusion does not seem to be justified. Observations made in later investigations (HIAT & SCHORR, 1951, GUTMAN & YU, 1951, NEUMAN, DISTEFANO & MULRYAN, 1951) give strong reason to question whether any local increase in the concentration of inorganic phosphorus occurs in the enchondral calcification; the role of the glycogenolytic cycle and alkaline phosphatase in the process of calcification must still be regarded us unclear. Neither is conclusive evidence available that vitamin D exerts a direct effect at the site of bone deposition. In their report on experiments in rachitic rats NICOLAYSEN & JANSEN (1939) pointed out that the degree of calcification depended upon the amount of calcium and phosphorus presented to the blood stream. In animals with vitamin D deficiency the histological structure was, however, abnormal. In experiments with rachitic dogs MELLANBY (1949) noticed that raising the vitamin intake from 20 I. U. to 1 000 I. U. daily improved the structure of bone without significantly altering the absorption of calcium. Judging by these 2 last-mentioned papers then, there seems to be a direct skeletal action of vitamin D in the correction of bone structure. The actual mechanism of this effect is, however, not yet properly understood.

By means of ordinary chemical methods it has not been possible separately to study the two fundamental processes involved in skeletal metabolism, namely the deposition and the removal of calcified tissue. The relative ash content in bone has hitherto been regarded mainly as a function of the deposition of bonesalt (cf. NICOLAYSEN & JANSEN, 1939), despite the fact that it has been stressed by histologists that the removal of bone can be observed in an early stage in healing rickets (DODDS & CAMERON, 1938). MIGICOWSKY & EMSLIE (1948) studied this problem with a tracer technique. They gave radiocalcium to rachitic chicks one week prior to the start of the

experiment with deposition of the absorbed Ca^{45} in the skeleton. In the experiments half of the animals received repeated doses of vitamin D. They found that the total calcium content and the specific activity of the calcium excreta were less in the vitamin D treated chicks than in the others. They therefore supposed that vitamin D functioned by inhibiting the solution of calcium from the bone. These authors, however, forgot to take into consideration an important point, namely that calcium removed from the bone is to a large extent taken up again by the skeleton and that only part of the calcium dissolved leaves the body with the excreta. This excreted quantity need not, of course, be proportional to the total amount of calcium removed from the skeleton. It is therefore not possible to draw any conclusions about the degree of bone resorption from the amount of Ca^{45} recovered from the excreta. Whether and in what direction vitamin D influences the process of removal of calcified tissue in healing rickets therefore seems to require further investigation.

The purpose of the present investigation was to study more closely the effect of vitamin D on the absorption of calcium from the intestinal tract by means of the tracer technique. As it was also possible to differentiate the deposition from the removal of calcium in calcified tissue in this way, attention was also directed to the course of these two processes in the healing stage of rickets.[1]

The interpretation of the uptake of radioactive isotopes in the skeleton has been the subject of much discussion. It has been claimed that P^{32} and Ca^{45} are taken up by the skeleton at a rate too quick to be ascribed to the formation of calcified tissue (MANLY, HODGE & MANLY, 1940, HARRISON & HARRISON, 1950). They therefore assume that a considerable part of this uptake must represent an exchange of Ca^{40} situated on the surface of the bone crystals, for Ca^{45} of the surrounding body fluids, i. e. what is generally called surface exchange. Surface

[1] A preliminary report of some of these experiments was presented in an earlier paper (LINDQUIST, 1951).

exchange has been demonstrated in *in vitro* experiments in which powdered bone ash was brought together with Ca^{45} or P^{32}-solutions and it has been computed that the percentage of isotope exchange is related to the size of the crystals and the surface area available for exchange (FALKENHEIM, NEUMAN & HODGE, 1947, A, B). The part of the skeletal calcium that can be regarded as situated on the surface of the crystals is about 12 per cent (HENDRICKS, 1951). The latter author stresses, however, that only a part of this is accessible to surface exchange and that it is unknown how large this part is. ROBINSON (1951) claims that computations about the isotopic exchange on the basis of the surface area of the crystals hold good only in bone from which the organic matter has been removed. NEUMAN & MULRYAN (1950) found in *in vitro* experiments with P^{32} that powdered unashed bone, especially from actively growing areas, undergoes a process of recrystallisation, i. e. a process of dissolution and reprecipitation of bone crystals. Later, however, NEUMAN (1951) pointed out that it is impossible to say to what extent this reaction *in vivo* can be due to the incorporation of new crystals in the bone salt, but added, that in rapidly growing animals this is not an unimportant factor. FALKENHEIM, UNDERWOOD & HODGE (1951) carried out similar experiments *in vitro* with Ca^{45}. But their results did not provide conclusive evidence of the presence of a recrystallisation process as regards the uptake of Ca^{45}. Whether and to what extent the uptake of Ca^{45} *in vivo* is too quick to be ascribed to the formation of calcified tissue is not known. CARLSSON (1951) found at an interval of 18 hours after the administration of radiocalcium the skeletal distribution of Ca^{45} to be governed by the rate of formation of calcified tissue in the different parts of the skeleton. Parenteral administration of phosphate to rachitic rats simultaneously given Ca^{45} *per os* (see chapter X) produces a prompt and pronounced uptake of Ca^{45} by the skeleton and a marked decrease in the excretion of radiocalcium with the urine. In this case the rapid uptake of radiocalcium in the bones cannot be accounted for by any other process than a deposition of calcium

salts, because in the corresponding control group that received no P the uptake of Ca^{45} was much smaller. In studies and computations of the calcification by the tracer technique the influence that removal of calcified tissue may exert, the rate and size of which nothing definite is known either, also seems to have been left out of consideration. In this connection it should be pointed out that resorption of bone is an integral part of skeletal growth and necessary for modelling the bone, a fact easiest to observe in the ends of the long bones (see WEIMANN & SICHER, 1947). On the basis of observations made in autoradiographic studies LEBLOND, WILKINSON, BELANGER & ROBICHON (1950) reported that in young rats the walls of the tibia shafts and the spicules distal to the epiphyseal plate at the head of the tibia renew themselves in an 8 day period. Thus there seems to be no relevant discrepancies in the interpretation between data obtained by tracer experiments and results as shown by morphological and ordinary chemical methods.

CHAPTER II

Experimental Conditions

Animals and Diets Used

The experiments were done on rats from a mixed strain that had been inbred for many years. Animals of both sexes were used.

During the first 25 days of life the rats lived together with their mothers, which were fed on Bills' diet (see BILL *et al.*, 1931). The animals were then kept in the dark and fed on a modification of Steenbock's rachitogenic diet Nr. 2965 (see COHN & GREENBERG, 1939). The diet was as follows:

Ground whole yellow corn	74
Pure gum gluten	20
$CaCO_3$	3.75
NaCl	1
Wheat germ	1

Analysis of the diet showed a Ca content of 1.48 per cent and a P content of 0.25 per cent, thus a Ca/P ratio of 5.9.

The animals were allowed to eat and drink ad libitum.

After having been fed on this diet for 21 days some of the animals received vitamin D in the form of a single dose of calciferol (British Drug Houses), the dose being varied according to the nature of the experiment. The vitamin was dissolved in peroxide-free peanut oil and administered via a stomach tube.

A number of control animals received a similar quantity of peanut oil only. The animals were deprived of food for 6 hours

before the administration of the vitamin and for 3 hours afterwards.

In one series the vitamin was given continuously while the animals were on the rachitogenic diet. These animals received 20 I.U. vitamin D in 0.1 ml. peanut oil every other day by means of a pipette.

In another experimental series the effect of an addition of P to the rachitogenic diet was studied. A quantity of 4.1 gm. NaH_2PO_4, $2H_2O$ was added per 100 gm. of the rachitogenic food. This changed the dietetic Ca/P ratio from 5.9 to 1.4.

In every experimental series the groups were as uniform as possible as regards litter, sex and body weight. Unless otherwise stated, the weights given in the tables are those recorded on the day the animals received the vitamin or the peanut oil.

Sampling Technique

After an interval varying from one experimental series to another — usually 4 days after the administration of calciferol or peanut oil — the animals received radiocalcium. Unless otherwise stated, the animals were deprived of food for 6 hours before this administration and for 3 hours afterwards. The radiocalcium was given in the form of an aqueous solution, and by means of a stomach tube or subcutaneously. Radiocalcium was given as the lactate in 20 mg. Ca when administered orally, and in 10 mg. when it was injected subcutaneously. When administered by the oral route the radiocalcium was given in 1.5 ml. fluid. Afterwards 0.5 ml. distilled water was introduced for rinsing the tube. When the radiocalcium was injected subcutaneously the total quantity of fluid administered was 1.0 ml.

After the administration of the radiocalcium each animal was placed in metabolism cage by itself. The cages had an arrangement for separating the faeces from the urine (see BORGLIN, 1947). In some of the experiments in which the animals were not killed until a relatively long time after they had received the radiocalcium and in which the excretion of the

isotope was not followed throughout the experiment, the animals were kept in ordinary cages. The animals were decapitated or bled under ether anaesthesia. In most of the animals the presence or absence of rickets was determined by roentgenography of the right tibia. In some cases the head of the tibia was also studied histologically.[1]

Different specimens were used for different experiments. When determing the activity of the serum calcium the blood of each group was first pooled. Various skeletal parts were removed. The left femur was treated as one specimen and the right as another. The mean of the values was said to be the value for the femur. The ends of both tibia-fibulae were cut off, pooled and regarded as a single sample. The rest of the tibia-fibulae (shafts) were also pooled and taken as a single sample, as were the four incisors. The faeces and the urine were collected separately, and the digestive tract of each animal was added to its faecal collection. When necessary, the urine-faeces separator was washed. As rests in the separator usually consisted of faeces, they were added to the fraction for the digestive tract + faeces.

The urinary samples and the washings were ashed until practically free from fluid, the ashing agents used being perchloric acid and nitric acid in equal proportion. The skeletal specimens and the samples of the digestive tract + faeces were dried and ashed at about 700° C in an electric muffle. Dilute hydrochloric acid was added to the silicious ash obtained from the digestive tract + faeces. This sample was then dried on a water bath. Dilute HNO_3 (density 1.10) was now added and the solution was filtered. All the other specimens were quantitatively dissolved in this solvent. Then, according to the degree of activity expected, the different specimens were made up with the dilute HNO_3 to volumes varying from 3.5 ml. to 50 ml. Tubes graduated in 0.1 ml. were used for dilution to volumes smaller than 10 ml.

[1] I am indebted to Bengt Gustafsson, M.D. for the histological examinations.

The serum calcium was determined by the method of CLARC & COLLIP (1925) and on samples pooled in each group. The inorganic P in the serum was determined by the method of YOUNGBURG & YOUNGBURG (1930). In most experiments the phosphorus determinations were made on unpooled samples.

Remarks on Radiocalcium

In the present study the Ca^{45} employed consisted of irradiated units supplied by HARWELL in the form of $CaCO_3$. The specific activity of this radiocalcium was given by the suppliers as 0.05—0.1 mC/gm. Ca. Because of radioactive contamination — which, according to Harwell, was, however, only very slight — the preparations were purified by the method described in 1947 by KAMEN (see CARLSSON, 1951).

The radioactivity of the specimens was compared with that of a standard solution of known Ca concentration and taken from the same irradiated unit as the Ca^{45} of the specimen. The radioactivity was determined with a Geiger-Müller tube of end-window type. The determinations were made on liquid samples. The tube and counter used and the arrangement for determining the radioactivity were the same as those described by CARLSSON (1951).

Statistics

The range of error of activity determinations recorded by the Geiger-Müller counter can be estimated theoretically. According to Poisson's law, the standard deviation of a single observation is equal to the square root of the number of counts observed. But there are, of course, also sources of error other than those referable to the chance variation in the emission of radioactive particles. This can be deduced *ad modum* DAHLBERG (1948) from double determinations. The results obtained are in accord with those reported by CARLSSON (1951). In the present study the standard deviation of a single observation accepted was at most 7 per cent. This was not applied so

strictly regarding certain specimens of the urine and washings with low radioactivity. Otherwise in the statistical treament of the data the procedures described by DAVIES (1949) and BLISS (1951) were used.

The standard error was calculated for most groups: in the tables it is given after the mean value, and in the figures as a column on both sides of the mean value.

CHAPTER III

Effect of a Varying Dose of Vitamin D on the Absorption and Skeletal Distribution of Radiocalcium in Rachitic Rats

In investigations on the mode of action of vitamin D the experimental animals often received heavy doses of the substance without it being stated whether the purpose of the experiments was to study the physiologic or the toxic effect of such medication. Toxic doses can produce effects not seen after the administration of physiologic doses. For instance, it requires a heavy dose of vitamin D to produce the effect reported by ALBRIGHT & SULKOWITCH (1938) of vitamin D on the metabolism of phosphorus, namely increased excretion of phosphorus with the urine. In kidney studies the use of large doses has been practically the rule (cf. HARRISON & HARRISON, 1941). NICOLAYSEN & NJAA (1951) stresses that as clear a line as possible should be drawn between the physiologic and the pharmacologic or toxic effect of vitamin D. As the purpose of the present investigation was mainly to study the effect of physiologic doses of vitamin D, it was important first to gain an idea of the relationship between the effect of vitamin D and the size of the dose administered. As evidence is available that vitamin D promotes the absorption of calcium from the intestine — directly or indirectly — it may be convenient at this stage to discuss this function of vitamin D. The skeletal uptake of radiocalcium was also studied in this connection.

Experiments

A dose of 20 mg. calcium lactate containing radiocalcium was administered by means of a stomach tube to rachitic rats, which were killed

18 hours later. The volume of fluid administered was 2 ml. Four days before the administration of the radiocalcium some of the animals received calciferol dissolved in peanut oil, likewise via a stomach tube, while the remainder, the controls, received peanut oil only. The experiment was carried out on 3 series of animals.

The first series was divided into 5 groups of 4—5 rats. Each of these animals received vitamin D in a dose varying from 0.5 I.U. to 1 000 I.U. The second series was likewise divided into 5 groups of 4—5 rats. These animals received a dose varying from 0.5 I.U. to 16 I.U. The third series comprised 3 groups, each of which consisted of 8 animals. The first group received peanut oil only, the second were treated with 15 I.U. and the third with 50 000 I.U. vitamin D.

Results

Table I and Figs. 1, 2, and 3 summarize the results. The fraction of the administered calcium that had been absorbed was calculated from the difference between the amount of radiocalcium given and of that recovered from the digestive tract + faeces at the end of the experimental period. The actual amount absorbed was, of course, higher on account of the fact that some of the radiocalcium absorbed returns into the intestine, but this difference was slight (see chapter VII). Therefore, the above mentioned expression for the absorption seems to be representative. As expected, the administration of vitamin D considerably increased the absorption of radiocalcium from the intestine. Table I (series I) shows that a dose of 10 I.U. vitamin D was sufficient to produce a practically maximal effect on the absorption of radiocalcium from the intestine. But a heavy dose produced a slight, further increase in the absorption (series III). The difference between the values obtained for the animals treated with 15 I.U. and 50 000 I.U. was almost statistically significant ($0.01 < P < 0.05$). The curve in Fig. 1 shows that the absorption of radiocalcium increased with increasing dose of vitamin D and that doses of 1 I.U.—8 I.U. produced the greatest effect per unit.

The groups of the respective series varied but slightly from one another as regards the excretion of radiocalcium with the urine.

Table I. *Effect of a varying dose of vitamin D on the absorption, urinary excretion and skeletal distribution of radiocalcium in rachitic rats.*

Exp. series nr.	Time after Ca^{45} hours	I. U. vitamin D	Nr. of rats	Body wt grams	Ca^{45} % of dose					
					Absorption	Urine	Femur	Incisors	Tibia-fibula ends	Tibia-fibula shafts
I	18	—	5	80	27.2 ±2.4	7.1 ±0.9	0.53 ±0.03	0.73 ±0.06	0.73	0.26
	18	0.5	4	79	33.7 ±2.8	7.8 ±0.4	0.63 ±0.02	0.87 ±0.08	0.79	0.34
	18	1	5	81	34.1 ±2.7	7.5 ±0.9	0.79 ±0.10	0.85 ±0.07	1.07	0.38
	18	10	5	81	48.3 ±2.2	8.2 ±0.3	1.41 ±0.11	0.95 ±0.05	2.44	0.48
	18	50	4	78	51.1 ±1.5	9.4 ±0.2	1.50 ±0.03	0.96 ±0.06	2.56	0.51
	18	100	4	75	51.2 ±3.4	8.9 ±1.5	1.42 ±0.17	0.99 ±0.04	2.39	0.48
III	6	—	3	71	31.8 ±2.1	5.3 ±0.2	0.69 ±0.03	0.65 ±0.02	1.04	0.34
	18	—	4	68	33.7 ±2.6	10.5	0.51 ±0.08	0.81 ±0.02	0.84	0.35
	6	15	4	71	48.5 ±0.8	5.4 ±0.1	1.60 ±0.08	0.77 ±0.05	2.82	0.42
	18	15	4	73	51.3 ±2.7	10.7 ±2.1	1.43 ±0.09	1.07 ±0.07	2.61	0.47
	6	50.000	4	74	57.0 ±2.8	3.6 ±1.2	2.03 ±0.21	0.78 ±0.04	3.61	0.47
	18	50.000	4	67	56.0 ±3.2	11.9 ±0.9	1.62 ±0.07	0.96 ±0.07	2.88	0.41

The skeletal uptake of radiocalcium also increased with an increasing dose of vitamin D up to a certain level, after which the uptake increased only slightly. This was clear from the curve of response of the femurs and the ends of the tibia-fibula (Figs. 2 and 3). Here, too, a heavy dose of vitamin D produced a further increase in the skeletal uptake of radiocalcium — the difference between the values for the femurs of the animals treated with 15 I.U. and of those that received 50 000 I.U. was almost statistically significant ($0.01 < P < 0.05$). The degree of the effect of vitamin D, however, varied widely from one part of the skeleton to another. Thus the increase in

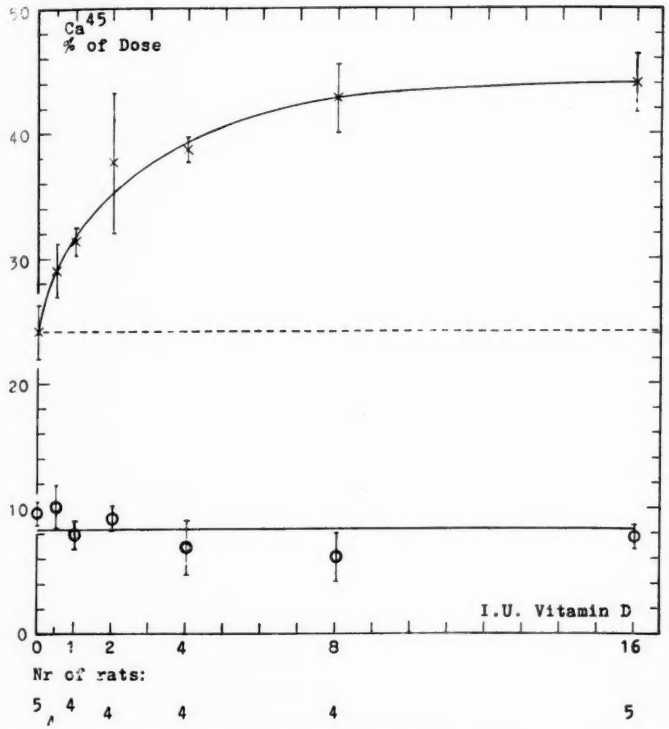

Fig. 1. Effect of a varying dose of vitamin D on the absorption and urinary excretion of radiocalcium in rachitic rats.
× Absorption
○ Urinary excretion

the uptake of radiocalcium by the shafts of the tibia—fibula was much smaller, and the increase in the uptake by the incisors was still smaller.

Discussion

Thus a dose of 15 I.U. produced practically maximal effect on the absorption of calcium from the digestive tract. There-

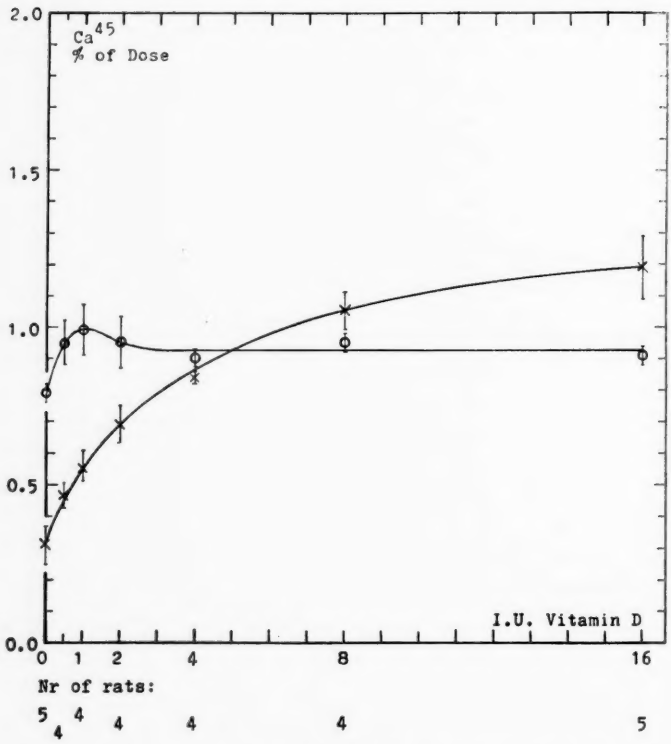

Fig. 2. Effect of a varying dose of vitamin D on the uptake of radiocalcium in the femur and incisors in rachitic rats.

× Femur

○ Incisors

fore, it was decided to use this dose in the study of the physiologic effect of vitamin D.

In the estimation of the skeletal uptake of radiocalcium consideration must be given to the specific activity of the serum calcium throughout the experiment, i. e. the average specific activity. The specific activity of the blood that could be assessed was, however, that at the end of the experiment, i. e. usually

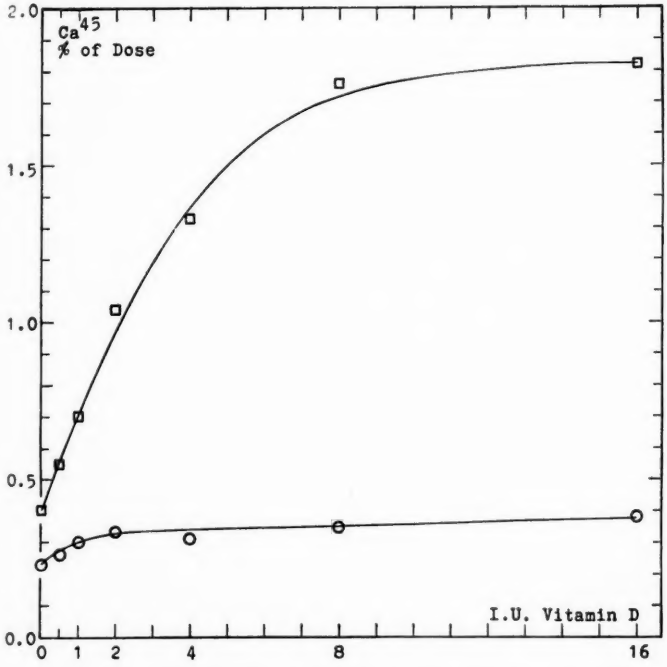

Fig. 3. Effect of a varying dose of vitamin D on the uptake of radiocalcium in the ends and shafts of tibia-fibula in rachitic rats.
□ Ends of tibia-fibula
○ Shafts of tibia-fibula

18 hours after the administration of the radiocalcium. At this stage no difference was found betwen the specific activity of the blood of the vitamin D treated and untreated animals. (In 13 consecutive series the mean value for the vitamin D treated animals was 0.298, and for the untreated it was 0.294, expressed as the percentage of the dose per milligram calcium. The difference between the two groups was 0.004 ± 0.011. The mean value for the serum calcium expressed as milligrams per 100 ml. was 11.2 ± 0.2 and 10.8 ± 0.3 respectively for the

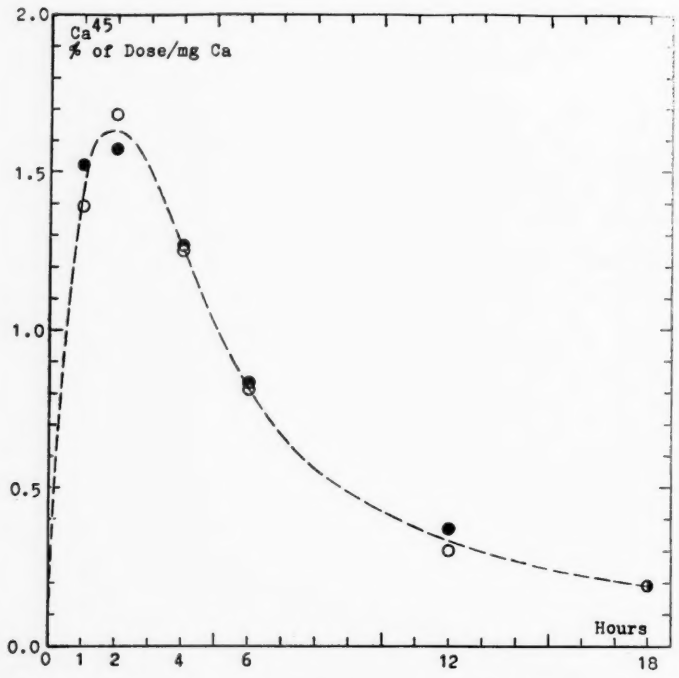

Fig. 4. The specific activity of the serum calcium at a varying interval after oral administration of radiocalcium to rachitic rats vitamin D treated (solid symbols) and untreated (open symbols).

same series.) As shown in Fig. 4 (as to the experimental conditions, see chapter VII) the specific activity of the serum calcium was also practically the same in both groups at other intervals after the oral administration of radiocalcium. In other words, there is reason to assume that the average specific activity of the blood is the same in the vitamin D treated animals as in the untreated ones. This means that the increased uptake of radiocalcium by the skeleton in the vitamin D treated animals really represents a true increase in the uptake of the calcium.

This lack of difference between the specific activity of the serum calcium of the vitamin D treated animals and the untreated ones calls for a few comments. The increased absorption of radiocalcium in the vitamin D treated rats ought to increase the specific blood activity in these animals. That no difference in specific blood activity could be demonstrated between these two groups of animals may be explained by the assumption that the radiocalcium leaves the blood quicker in the vitamin D treated ones than in the others. But this can hardly be the true explanation, for it must be assumed that Ca^{45} and Ca^{40} are taken up by the skeleton in the same proportion as they are present in the serum. Ca^{45} and Ca^{40} are also excreted in the same proportion with the urine as they are present in the serum. (GOVAERTS, 1949). The other possibility is that this absence of difference may be ascribed to an increased passage of inactive calcium from the bones into the bloodstream in animals treated with vitamin D. This explanation is in accord with results obtained by CARLSSON (1952 C) under experimental conditions different from those used here.

This observation that the specific activity of the serum calcium is not affected by vitamin D may not be generalised, and it is possible that differences may occur under experimental conditions other than those employed in the present experiments.

MELLANBY (1949) stressed that more vitamin D may be required to ensure perfect bone calcification than to produce maximum Ca absorption. The experiments described in this chapter did not contribute to our knowledge of the *relative* effect of varying doses on the absorption of calcium from the intestine and on the uptake of calcium by the skeleton. Such a study requires more detailed experiments in which the average specific activity of the serum calcium is determined after every dose.

Summary

The effect of the variation of the dose of vitamin D on the absorption and skeletal distribution of radiocalcium given orally

as the lactate was studied 18 hours after the isotope had been administered.

Both the absorption and the skeletal uptake of radiocalcium increased with increasing dose of vitamin D up to about 15 I.U., after which there was only a slight increase of the effect.

The increase in the skeletal uptake of radiocalcium in rachitic rats treated with vitamin D must reasonably represent an increased deposition of calcium salts.

The administration of vitamin D did not increase the specific activity of the serum calcium despite an increase in the quantity of radiocalcium entering the bloodstream from the intestine. The most reasonable explanation for this phenomenon may be that the vitamin D simultaneously increases the amounts of inactive calcium entering the blood stream from the bones.

CHAPTER IV

The Skeletal Uptake of Radiocalcium in Rachitic Rats as a Biological Assay for Vitamin D

If a varying dose of vitamin D is administered at a constant interval before the administration of a dose of radiocalcium, the skeletal uptake of radiocalcium, as measured by the uptake in the femur and ends of the tibia-fibula, will give a curve of response that is obviously logarithmic throughout the major part of its length (Fig. 5, corresponding to series II chapter III). This means that a dose-response curve, in which the effect of vitamin D is determined by the skeletal uptake of radiocalcium, can be used as a test for vitamin D. SNYDER, EISNER & STEENBOCK (1949) give a brief description of a method for testing vitamin D which was based on the assessment of the degree of radioactivity after intraperitoneal administration of P^{32}. The degree of activity was measured as an external count over the forepaw of the rat 8—10 days after the administration of vitamin D and of P^{32}, given shortly after one another.

The purpose of the following experiments was to exemplify the assessment of the vitamin D potency in an oil by means of a curve of response, where the effect of the vitamin is determined as the skeletal uptake of radiocalcium.

Experiments

Rats with experimental rickets produced in the manner described in chapter II were used. Calciferol dissolved in peanut oil was used as a test substance. An oil solution of vitamin D_3 containing 10 I.U./ml. was employed as a reference oil. The dose of test and reference oil given was

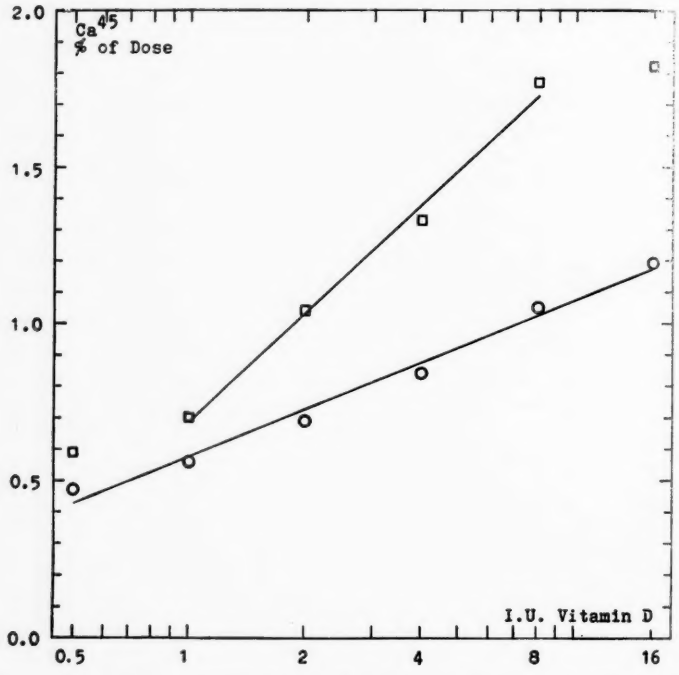

Fig. 5. Relationship between a varying dose of vitamin D (log. scale) and the uptake of radiocalcium in the femur and ends of tibia-fibula.

○ Femur
□ Ends of tibia-fibula

made up to 0.5 ml. with peanut oil. The dose was administered by means of a stomach tube and a tuberculin syringe four days before the administration of the radiocalcium. This was given as the lactate in a dose of 20 mg. calcium likewise via a stomach tube. As to starvation of the animals before and after the administration of vitamin D and of Ca^{45}, the experimental conditions were the same as those described in chapter II. The animals were killed 18 hours after the administration of the radiocalcium and the ends of the tibia-fibula were examined for radioactivity.

The animals were divided into 4 groups, 2 of which received test oil and 2 reference oil. The proportion between the size of the two doses of both test oil and reference oil was 1:3. Thus the two groups treated

Table II. *The uptake of radiocalcium in the ends of the tibia-fibula of rachitic rats after administration of test substance and reference oil.*

Test substance		Reference oil	
Tibia-fibula ends 0.1 ml	Tibia-fibula ends 0.3 ml	Tibia-fibula ends 0.1 ml	Tibia-fibula ends 0.3 ml
0.96	1.77	0.94	1.57
1.18	1.87	0.96	1.70
1.30	1.68	1.14	1.41
1.12	1.95	1.01	1.42
1.25	1.72	1.08	1.30

with reference oil received 1 and 3 I.U. vitamin D, respectively. In order to find out whether the experiment was arranged within a convenient range of the curve of response, one group of animals received only peanut oil under the same experimental conditions (negative control) and another group received 15 I.U. vitamin D (positive control). The groups were as uniform as possible regarding litter, sex and weight.

Results

The activity determinations of the test substance and reference oil made in this experiment are given in detail in table II. The figures denote the percentage of the radiocalcium recovered from the skeletal parts. The mean determination for the negative controls was 0.72 and for the positive controls 2.50. This means that the doses of test oil and of reference oil lay within the intermediate zone of the curve of response.

The equation for the regression line calculated from the values for the test substance was $y = 1.3286\,x + 1.1626$, in which y denotes the Ca^{45} uptake and x the log dose. The corresponding equation for the reference oil was $y = 0.9473\,x + 1.0267$. The log potency of the test substance relative to the reference oil (M) was then calculated from the formula $M = \frac{\bar{Y}_t - \bar{Y}_r}{b_c}$ where b_c denotes the regression coefficient for the combined line of the both oils, and Y_t and Y_r the values of y in

the regression lines for the mean values of x (see György, 1951). In this way M was calculated as 0.1994. The antilog for this value gives the relationship between the strength of the test substance and reference oil. The potency of the test substance was thus found to be 16 I.U./ml.

The standard error of the slope for the test substance and reference oil was found to be 0.1678 and 0.1699 respectively, which means that each slope was significant ($P < 0.001$). It is then necessary to determine whether the two oils differ significantly in slope, which would indicate a qualitatively difference between the two substances. This was calculated by analysis of variance. As the error variances for the two lines agreed very well, the sum of squares about each line were pooled to obtain the error variance about both curves ($s^2 = 0.01621$) with $DF = 16$. The mean square for the divergence in slope of the two oils was 0.04120 ($DF = 1$). The variance ratio was then calculated and found to be 2.54, which is smaller than that necessary for significance ($F = 4.49$; $P = 0.05$). The effect of the combined slope was highly significant ($F = 90.7$; $DF = 1$). The mean response to the test and reference oil was also compared in the same analysis; the mean square was found to be 0.2576 ($DF = 1$). The F ratio (15.9) indicated a high significance.

The standard deviation of the assay as measured into units of log doses (λ) is $= \frac{s}{b_c} = 0.11187$. This value is generally used as a criterion for comparing alternative methods: the smaller λ, the greater is the inherent precision of an assay technique. In the present investigation λ was relatively low (see GYÖRGY, 1951).

The standard error of M (s_M) was according to BLISS (1951) computed by the formula

$$s_M = \lambda \sqrt{\frac{1}{N_t} + \frac{1}{N_r} + \frac{(\bar{Y}_t - \bar{Y}_r)^2}{B_c^2 - t^2 \cdot s^2}} = 0.0546$$

where N_t and N_r is the total number of observations in each group. B_c^2 denotes $\frac{\Sigma^2(x-\bar{x})(y-\bar{y})}{\Sigma(x-\bar{x})^2}$, and $t = 2{,}92$ ($P = 0.01$; $DF = 16$). The antilog of s_M was 1.134. The percentage standard error was then 13.4. The approximate limits were computed from $M \pm t \cdot s_M$ ($t = 2.12$ for $P = 0.05$ and $DF = 16$) i. e. $0.3152 - 0.0836$, the antilog of which is 2.065 and 1.213. Therefore the true potency will fall within 21 I.U./ml. and 12 I.U./ml.

Discussion

If the degree of calcification produced by the administration of graded doses of vitamin D is determined by the line test or roentgenographically, the curve of response will also be of logarithmic type (COWARD, 1947, GYÖRGY, 1951). Most of the biological assays of vitamin D described earlier are based on a comparison between the degree of calcification of the metaphysis, as measured in this manner, with degrees given on a standard scale. It is, however, obviously difficult accurately to assess the degree of healing in a given bone in this manner, especially when healing is atypical (cf. KRAMER, SHEAR & SIEGEL, 1931 B) and this difficulty provides one of the greatest sources of error of these methods. In the method of determination used in the present experiments, in which the response to vitamin D was measured by the skeletal distribution of radiocalcium, the values obtained are also relative measures of the degree of calcification. An advantage of this method is, however, that the reading of the response of the skeleton to the vitamin excludes a personal source of error. As the vitamin D requirement of rats varies from time to time, it is necessary — as in other biological assays of vitamin D — to administer reference oil to a parallel group of animals every time a determination is made.

In the present investigation only the skeletal uptake of radiocalcium was analysed. Another possibility would have been to use the absorption of radiocalcium from the intestine as a test, as suggested by CARLSSON (1952 B). Each method has its advantages. The advantage of using the uptake of radiocalcium in the bones as a test is that the vitamin D produces a relatively greater response in the bones than in the intestines. However, if the test substance contains phosphorus, measurement of the effect of vitamin D by the skeletal uptake of radiocalcium is less convenient (cf. chapter IX). In such cases, however, the absorption method can be used without the phosphorus content of the test substance influencing the results.

Summary

It was shown that the response of a rachitic rat to a dose of radioactive calcium is quantitatively related to the dose of vitamin D given, and that the shape of the curve for the uptake of radiocalcium in the bones is logarithmic. A biological assay for vitamin D was devised on the basis of this observation. According to this assay, the response to a given dose of vitamin D is recorded as a measurement of the radioactivity in the ends of the tibia-fibula 18 hours after the administration of the radiocalcium.

CHAPTER V

Effect of Variation in the Interval between the Administration of Vitamin D and Ca^{45} on the Absorption and Skeletal Distribution of the Radiocalcium

In the discussion of the mode of action of vitamin D it has generally been conceived that no influence of the vitamin is demonstrable in bone until after a latency, and on the basis hereof certain theories have been put forward. IRVING (1944), for example, stated that the vitamin requires a definite interval of time for the mobilization of Ca and P, which state has to be attained in the body before vitamin action begins; once it has been reached, it exerts a maximal effect whether it begins early or late. IRVING (1944, 1946) found an optimal dose of vitamin D (20—30 I.U.) to produce an effect on the bones within 4—6 days. MORGAREIDGE & MANLY (1939), who used radioactive phosphorus recorded an interval of 54—72 hours before any effect of vitamin D could be demonstrated in the skeleton. They therefore supposed that certain intermediary processes are necessary to mobilize the phosphorus in a form suitable for deposition of bone salts. KRAMER, SHEAR & SIEGEL (1931 A) found, 3—4 days after healing of rickets was induced by cod liver oil, the appearance of new calcification in the tibia.

A perusal of the literature showed that this generally accepted conception of a latency in the action of vitamin D is based on less accurate quantitative methods, e. g. line test and histologic and roentgenographic observations. As it has proved possible to measure the effect of vitamin D more accurately with the aid of radioactive isotopes (chapter III), it was considered justified to study the problem with the technique described.

A brief report of the results of this study has been published earlier (LINDQUIST, 1951). Later, in 1951, UNDERWOOD, FISCH and HODGE also studied these questions. In their experiments they administered vitamin D and radiocalcium simultaneously. But this meant the bringing together of 2 variables. As the amount of radiocalcium in the skeleton also varies with the length of the interval after its administration (chapter VIII), the skeletal distribution of the radiocalcium will, under such conditions, be in different stages when variations in the time of action of the vitamin D is investigated. This is readily demonstrable in the study of the incisors.

The purpose of the experiments described below was to find out how quickly the effect of a physiologic dose of vitamin D can be observed by the absorption and skeletal distribution of radiocalcium, and how long this effect persists. In addition, a series was used to compare the effect of vitamin D administered as a single dose to animals with rickets and administered in the form of continual doses while the animals were fed on the rachitogenic diet. It will be noted that under these different conditions the structure of the skeleton differs. This can be seen in roentgenograms (Fig. 7, corresponding to experimental series nr. IV): in the first cases a rachitic bone is seen in the healing stage, in the second, the calcification zone is normal.

Experiments

Four series of rats were used. Each of the animals received 20 mg. radiocalcium as the lactate by the oral route and all were killed 18 hours later. The volume of fluid administered was 2 ml. The vitamin was given in 0.5 ml. peanut oil via the stomach tube at a varying interval before the administration of the radiocalcium. The control groups received 0.5 ml. peanut oil only.

In the first series the vitamin — 15 I.U. — was administered shortly (4—12 hours) before the radiocalcium. In the second series the interval was longer (more than 12 hours). This series comprised 4 subgroups: in 2 series the animals received 15 I.U. of D_2, and in 2 series 100 I.U. of D_2. In the third series the calciferol was dissolved in a 50 per cent solution of alcohol instead of in peanut oil but given under otherwise equal con

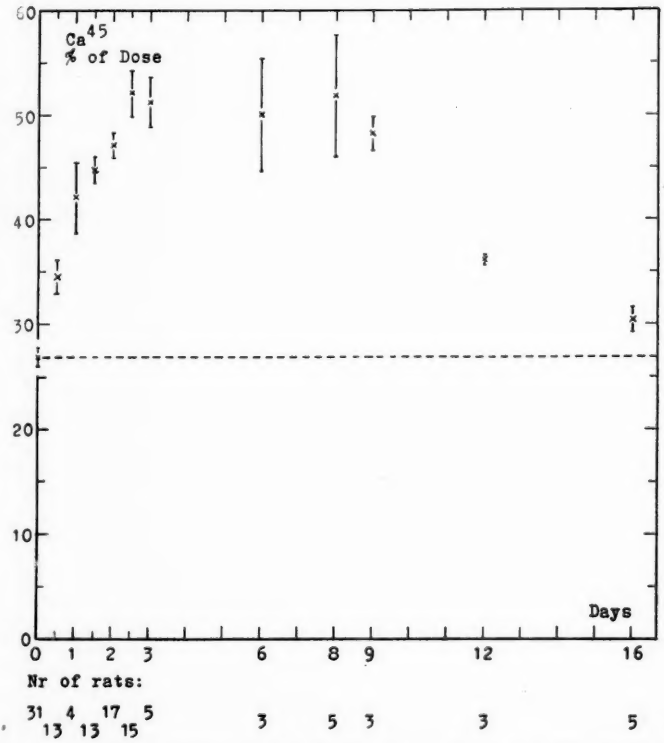

Fig. 7. Effect of variation in the interval between the administration of vitamin D and Ca^{45} on the absorption of the radiocalcium.

ditions. The quantity of vitamin administered was 15 I.U. In the fourth series the animals received 20 I.U. every other day as long as they were fed on a rachitogenic diet.

Results

The results are summarized in Figs. 7, 8, 9 and 10 and in tables III, IV and V.

Absorption and Excretion of Radiocalcium. A definite increase in the absorption of radiocalcium from the intestine was

Table III. *Effect of variation in the interval between the administration of vitamin D and Ca^{45} on the absorption and skeletal distribution of the radiocalcium.*

In series I the vitamin was administered in peanut oil.
In series III the vitamin was dissolved in 50 % alcohol.

Exp. series nr.	Vitamin D	Interval vitamin D—Ca^{45} hours	Nr. of rats	Body wt grams	Ca^{45} % of dose				
					Absorption	Femur	Incisors	Tibia-fibula ends	Tibia-fibula shafts
I	+	4	4	75	35.0 ±1.4	0.64 ±0.03	0.99 ±0.04	0.94	0.34
	+	8	4	76	31.4 ±1.4	0.69 ±0.06	0.92 ±0.05	0.93	0.32
	+	12	4	76	33.0 ±0.5	0.69 ±0.08	0.93 ±0.03	1.03	0.33
	—	12	4	73	27.7 ±1.2	0.51 ±0.04	0.78 ±0.05	0.69	0.28
III	+	24	5	65	33.6 ±1.6	0.59 ±0.04	1.30 ±0.04	0.84	0.38
	+	48	5	65	42.7 ±3.1	0.77 ±0.05	1.27 ±0.05	1.07	0.45
	+	72	5	65	46.9 ±3.6	1.04 ±0.04	1.29 ±0.05	1.48	0.47
	—	48	5	65	18.2 ±2.1	0.35 ±0.02	0.90 ±0.07	0.49	0.22

noticed already at an interval of 12 hours between the administration of the vitamin and of the radiocalcium (Fig. 7). In the animals treated with vitamin D within an interval of 12 hours (table III) and taken as a single group, the analysis of variance showed a statistically significant effect on the Ca^{45} absorption ($P < 0.01$). But no such difference was found between the 4.8 and 12 hours groups. The absorption then gradually increased with increasing interval (Fig. 7) and reached a maximum at an interval of about 72 hours, by when it was 51.2 per cent of the dose administered, which, as compared with the untreated animals (26.0 per cent), meant an increase of 90 per cent. At an interval of about 9 days the effect of the vitamin began to diminish and 16 days after the administration of the vitamin it had practically ceased.

Table IV. *The absorption, excretion and skeletal distribution of radiocalcium in rachitic rats (A), in vitamin D treated rachitic rats (B) and in rats that had been fed on the same rachitogenic diet but supplemented with vitamin D (C).*

	Nr. of rats	Body wt grams		Ash, mg				Ca^{45} % of dose					
		Initial	Final	Femur	Incisors	Tibia-fibula ends	Tibia-fibula shafts	Absorption	Urine	Femur	Incisors	Tibia-fibula ends	Tibia-fibula shafts
A	5	63	82	42	102	49	46	25.9	7.5	0.60	0.72	0.86	0.27
B	5	62	83	48	107	60	48	49.4	12.5	1.56	0.96	2.46	0.41
C	5	62	83	76	114	78	67	41.4	11.5	1.51	0.93	2.00	0.49

It was noted that the absorption of radiocalcium after a single dose of vitamin D seemed to be practically the same as after the prophylactic administration of the vitamin (table IV).

In rachitic rats a considerable portion of the dose administered orally is excreted with the urine, as compared with normal animals or animals fed on a calciumpoor diet (CARLSSON, 1951). In series II 10.0 per cent of the dose was excreted with the urine from the untreated animals, the corresponding percentages for the animals treated with the vitamin being 8.2, 8.1, 8.5, 10.7, 11.1, 10.5 and 11.8 at intervals of 12, 24, 36, 48, 60 and 72 hours, respectively. Thus the vitamin D treated animals did not differ with certainty from the untreated animals in this respect. As radiocalcium appears to be an equal proportion of calcium in the serum and in the urine (GOVAERTS, 1949), it may be concluded that the two groups did not differ from one another as regards the excretion of calcium, at least not under conditions in which the specific activity of the serum calcium is unaffected by vitamin D.

Radiocalcium in Calcified Tissue. The values for the first 12 hours are summarized in table III. As to the uptake of Ca^{45} in the femur at intervals of 4—12 hours the difference between the vitamin D treated and untreated animals was not so definite as was the difference in absorption at corresponding intervals. A tendency was, however, observed ($0.05 > P > 0.01$). The curve for the uptake of radiocalcium in the femur (Fig. 8) like that for absorption rose rapidly during the first 3 days and then

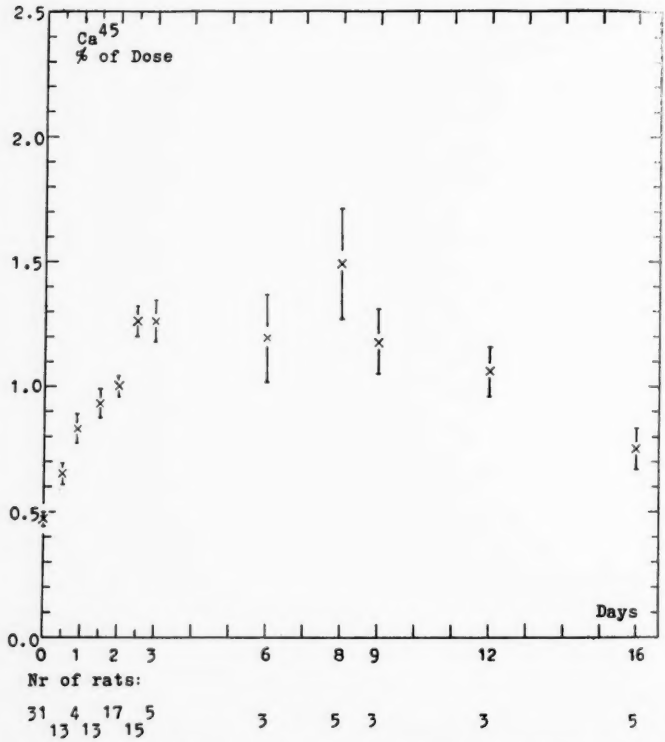

Fig. 8. Effect of variation in the interval between the administration of vitamin D and Ca^{45} on the uptake of the radiocalcium in the femur.

merged into a plateau. At an interval of 9 days the uptake of the radiocalcium began gradually to decrease. In the ends of the tibia-fibula (Fig. 10) the initial stage of the curve was steep, while the curve for the tibia-fibula shafts was flatter throughout the entire length of its course.

The effect of vitamin D on the uptake of radiocalcium in the incisors was observable already 12 hours after the administration of the vitamin (Fig. 9). The incisors were found to take up the maximum percentage of a given dose of radiocalcium

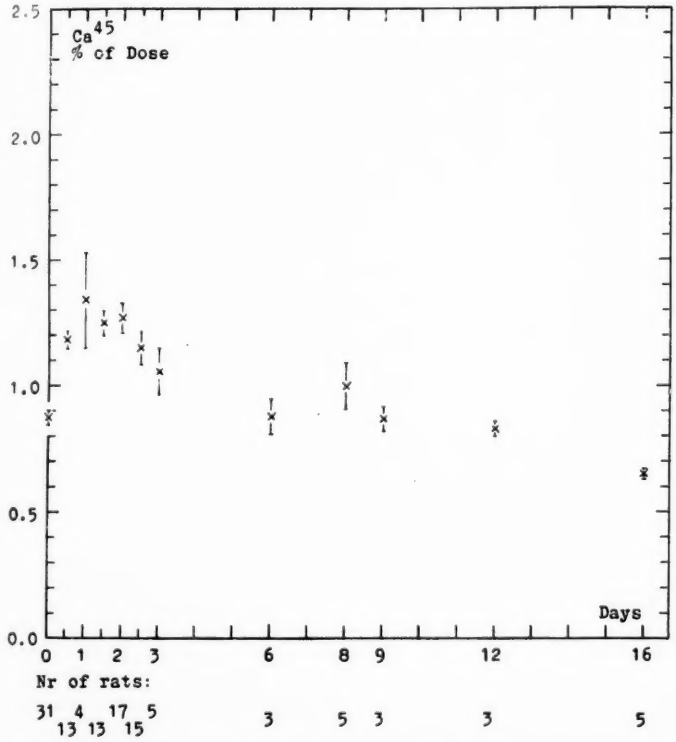

Fig. 9. Effect of variation in the interval between the administration of vitamin D and Ca^{45} on the uptake of the radiocalcium in the incisors.

when the vitamin was administered 24—48 hours before the Ca^{45}, i. e. before the vitamin exerts its full effect on the bones. When the vitamin was given a longer time before the administration of Ca^{45} the percentage of the isotope taken up by the incisors was smaller. The analysis of variance showed that the maximum values obtained at intervals of 24—48 hours differed statistically from a group of values recorded at bordering intervals (0—12 and 60—72 hours.).

A single dose of vitamin D had a greater effect on the uptake

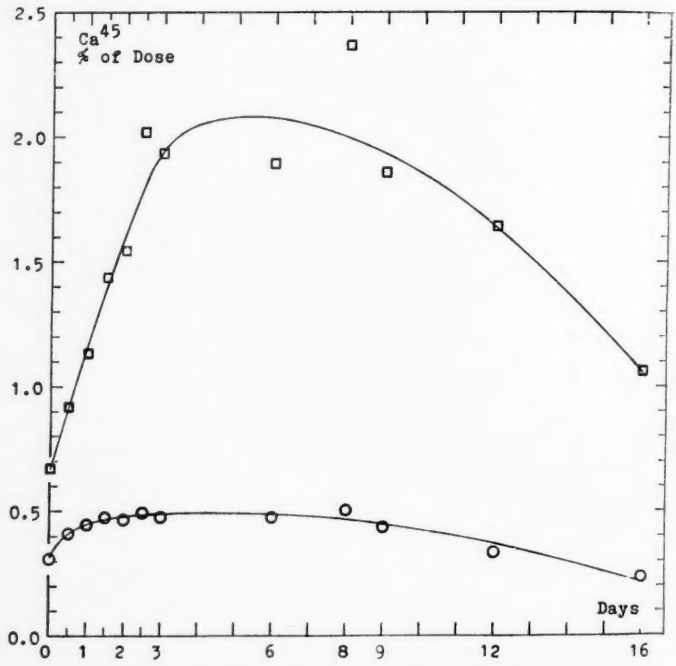

Fig. 10. Effect of variation in the interval between the administration of vitamin D and Ca^{45} on the uptake of the radiocalcium in the ends and shafts of the tibia-fibula.

□ Ends of tibia-fibula
○ Shafts of tibia-fibula

of radiocalcium in the ends of the tibia-fibula of rachitic rats than did continual administration. The interpretation of this finding is, however, complicated by the fact that no corresponding difference was found in the femur.

Ca and P in the Serum. In one subgroup belonging to series II determinations of the blood were also made (table V). In these experiments the level of the serum calcium seemed to be lower in the untreated than in those treated with vitamin D. The

Table V. *Effect of vitamin D on the serum Ca and inorganic P at a varying interval between the administration of the vitamin and orally given radiocalcium.*

Vitamin D	Interval vitamin D—Ca^{45} hours	Nr. of rats	Body wt grams	Serum		
				Ca^{45} % of dose per mg Ca	mg % Ca	mg % P
–	36–72	9	77.6	0.27	9.2	1.7
+	36	5	81.2	0.26	11.7	3.5
+	48	5	84.4	0.28	12.1	3.8
+	60	5	77.7	0.34	11.8	4.0
+	72	5	83.8	0.32	12.2	4.3
+	192	5	79.8	0.58	9.7	5.5
+	384	5	82.4	0.24	9.6	3.4

values recorded were in accord with those reported by KRAMER, SHEAR & SIEGEL (1931 A). The level of the serum inorganic phosphorus rose continously with increasing interval between the administration of vitamin D and radiocalcium. The results were in agreement with data reported by WARKANY (1935), who under similar conditions found a gradual increase in the inorganic phosphorus in rats after the administration of a single dose of viosterol. In contradistinction to the observation made in his experiments, the inorganic phosphorus in the present experiments decreased at longer intervals, but this difference is probably ascribable to a difference in the size of the dose of vitamin D given.

There was a close correlation between the inorganic phosphorus in the serum and the Ca^{45} uptake in the femur. The coefficient of correlation was 0.717 which is highly significant ($P<0.001$). The equation for the regression line was $y=0.232x+0.17$ in which $x=$ mg % inorganic P and $y=Ca^{45}$ uptake.

Discussion

The observations made with respect to the absorption and skeletal distribution of the radiocalcium argue against the assumption of a latency in the action of vitamin D and suggest

that it has a gradually increasing effect demonstrable after a fairly short time. It should be stressed that this gradually increasing effect also applies to the absorption from the intestine. As it did not sound unreasonable to suppose that this insidious effect might be ascribed to the oil in which the vitamin was dissolved in these experiments, the latter were repeated on another series, but this time the calciferol was dissolved in alcohol instead. REED (1950) pointed out that in experiments using heavy doses of vitamin D the vehicle in which the vitamin is given is of considerable importance, preparations dissolved in alcohol or propylene glycol appearing to be more toxic than do those dissolved in oil. But in the present investigation practically no difference was observed between the shape of the curve for the radiocalcium fractions using oil or alcohol, as a vehicle for vitamin D. The gradual onset of the effect may be interpreted as a suggestion that the vitamin D in turn builds up some regulating mechanism. Such mechanism may possibly be hormonal (NICOLAYSEN, 1943) or enzymatic. In *in vitro* experiments ZETTERSTRÖM (1951) showed that the administration of phosphorylated vitamin D increased the phosphatase activity.

The short interval between the administration of vitamin D and Ca^{45} necessary for the incisors to take up the maximum amount of radiocalcium might suggest that the calcium deficiency of the rachitic incisors was controlled within 24—48 hours. A possibly explanation for this fairly quick replenishment may be that in rachitic rats the mineralization in the incisors is less affected than the rest of the skeleton. This assumption is supported by the observation that the ash weight of the incisors of rachitic rats is only slightly lower than in normal animals. GAUNT, IRVING & THOMPSON (1939) also claimed that under unfavourable circumstances calcification is not disturbed to such a degree in the teeth as in the bones. This observation has been confirmed in rickets induced by a diet low in phosphorus and high in calcium content (see LEICESTER 1949). It is, however, remarkable that the Ca^{45} values for the incisors at an interval of 6 days or more are not higher than

those for the untrated rats despite the fact that one must assume that the deposition of calcium in the incisors is somewhat greater in the vitamin D treated animals than in the untreated ones. This may tentatively be explained by the assumption that the Ca deposition in the incisors at this interval to a relatively large extent consists of inactive calcium.

The fact that the urinary excretion of radiocalcium is practically the same in the vitamin D treated rats and in the untreated ones may be ascribed to a connection between the high calcium and low phosphorus content of the diet (KARELITZ & SHOL, 1927). On the basis of experiments with radiocalcium on "renal serum clearance" COPP, HAMILTON, JONES, THOMPSON and CRAMER (1951) suppose that the high urinary excretion is dependent on a direct effect on the calcium clearance by the kidney, probably by a decrease of re-absorption by the tubules.

Summary

It was shown that the effect of a physiologic dose of vitamin D on the absorption and skeletal distribution of radiocalcium was observed already at an interval of 4—12 hours between the administration of the vitamin and radiocalcium. The effect then increased and reached a maximum at an interval of about 60—72 hours, after which the curve merged into a plateau, begins gradually to fall at an interval of 9 days, and had practically ceased after altogether 16 days. The observations made argue against the earlier assumption of a latency in the action of vitamin D and suggest that it has a gradually increasing effect demonstrable after a fairly short interval.

The incisors were found to take up the maximum percentage of the radiocalcium administered when the vitamin was given already 24—48 hours before the radiocalcium, probably because the mineralization of the incisors is less affected than the rest of the skeleton.

It was noted that the absorption and skeletal distribution of radiocalcium after a single dose of vitamin D to rachitic rats seemed to be practically the same as after the prophylactic administration of the vitamin.

CHAPTER VI

Effect of Vitamin D on the Excretion and Skeletal Distribution of Radiocalcium after Parenteral Administration of the Isotope

In an investigation on the mode of action of vitamin D in rickets GREENBERG (1945) found that animals injected intraperitoneally with radiocalcium, during the healing stage accumulated almost twice as much radiocalcium per gram of dry bone and about 1.5 times as much per gram of bone ash as the untreated ones. GREENBERG stated further: "If the hypothesis that the only action of vitamin D is to promote absorption of calcium from the digestive tract were correct, the rachitic animals should retain as much of the injected Ca^{45} in the bones as do the vitamin D treated rats." This was not found to be the case. This finding was used as evidence that vitamin D has a direct action on the mineralization of bone in rachitic animals.

The problem was re-investigated, partly because studies using radioactive calcium under these conditions should also include a study of the specific activity of the serum Ca as well as the concentration of the total calcium and inorganic phosphorus in the serum, and partly because GREENBERG'S series was too small to permit any definite conclusions. Another purpose of the investigation was to study whether vitamin D exerts an effect on the passage of calcium into the digestive tract.

Experiments

The experiments were carried out on 2 series. In the first series 15 I.U. vitamin D was administered by means of a stomach tube at an interval

Table VI. *The specific activity of the serumcalcium at a varying interval after the subcutaneous administration of radiocalcium to vitamin D treated and untreated rachitic rats.*

Vitamin D	Ca^{45} % of dose per mg Ca after			
	1 hour	6 hours	12 hours	18 hours
–	2.67	2.42	1.66	0.98
+	2.34	2.08	1.19	0.60

varying from 12 to 72 hours before the administration of the radiocalcium. The control animals were given 0.5 ml. peanut oil only. The animals received 10 mg. radiocalcium as the lactate subcutaneously and were killed 18 hours afterwards. The volume of the fluid thus administered was 1 ml.

In the second series the vitamin was given at a constant interval throughout — 72 hours before the administration of the radiocalcium. The animals received 10 mg. radiocalcium in the same manner as in series I and were killed at an interval varying from 1 to 18 hours after the administration of the radiocalcium. In this series only blood determinations were made.

Results

The results are summarized in tables VI and VII and in Fig 11. The quantity of radiocalcium recovered from the digestive tract + faeces was largest in the untreated group. In the vitamin D treated groups it decreased with increasing interval between the administration of the vitamin and of the radiocalcium. The level of the urinary radiocalcium was higher throughout after the parenteral administration of the radiocalcium than after oral administration. Almost 20 per cent of the dose given was recovered from the rachitic animals, while the elimination was much less in the vitamin D treated animals. Although the urinary elimination of radiocalcium was higher throughout in GREENBERG'S (1945) series, the difference per cent between the urinary excretion of radiocalcium in the vitamin D treated and the untreated groups was the same as that observed in the present experiments. On the other hand, he found no difference between treated and untreated animals regarding the amount of radiocalcium recovered from the digestive tract + faeces.

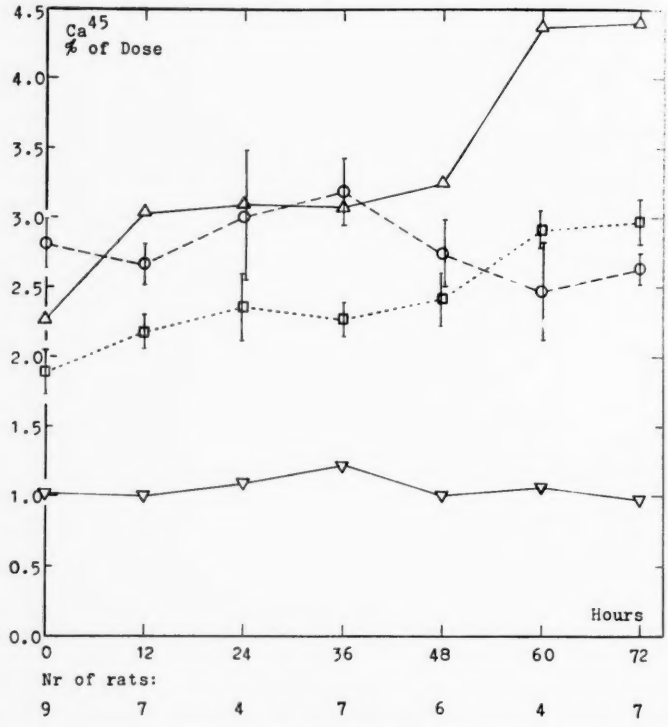

Fig. 11. Effect of vitamin D on the skeletal distribution of radiocalcium injected subcutaneously at a varying interval after the administration of the vitamin.

□ Femur
○ Incisors
△ Ends of tibia-fibula
△ Shafts of tibia-fibula

The uptake of radiocalcium in the femur and in the ends of the tibia-fibula increased somewhat irregularly with increasing interval after the administration of vitamin D (Fig. 11), but the difference observed at an interval of 60—72 hours was statistically significant ($P < 0.001$). The uptake of radio-

Table VII. *Effect of vitamin D on the distribution of subcutaneously injected radiocalcium in the digestive tract+feces and urine and on the values of serum Ca and inorganic P, as observed at a varying interval after the administration of the vitamin.*

Vitamin D	Interval vitamin D—Ca^{45} hours	Nr. of rats	Body wt grams	Ca^{45} % of dose		Serum		
				Digestive tract+feces	Urine	Ca^{45} % dose per mg Ca	mg % Ca	mg % P [1]
–	12–60	9	75	11.6±1.0	19.1±2.3	1.02	11.1	2.3
+	12	7	75	8.8±0.8	12.3±3.1	0.85	10.9	2.6
+	24	4	77	5.3±0.4	11.8±3.7	(0.57)	(16.4[2])	3.7
+	36	7	75	5.9±0.7	13.2±3.1	0.71	10.4	3.9
+	48	6	76	5.0±0.7	12.8±2.5	0.70	9.4	3.7
+	60	4	78	4.4±0.6	11.8±1.5	0.60	10.2	3.9
+	72	7	75	3.3±0.7	10.7±1.9	0.66	11.6	5.1

calcium in the shafts of the tibia-fibula was practically unchanged. As was the case with the oral administration under otherwise similar conditions (chapter V), the uptake of radiocalcium by the incisors reached a maximum at an interval of 24—36 hours, i. e. before the vitamin exerts its full effect on the bones. As the interval increased the quantity of radiocalcium recovered from the incisors decreased, probably on account of a decrease in the specific activity of the serum calcium (table VII).

The results obtained in series II are summarized in table VI. The specific activity of the serum calcium at a varying interval after the administration of radiocalcium was higher throughout in the untreated animals. The values decreased on the whole at the same rate as the interval increased. This difference was presumbably due to a greater passage of the calcium from the bones to the blood in the vitamin D treated animals (see chapter III). As before, the level of the serum calcium varied but slightly (table VII). The rise in the level of the inorganic phosphorus of the serum was slightly irregular,

[1] Mean of 7 animals in the untreated group and 4 animals in the treated groups.

[2] This high value is probably due to a technical error.

but it rose throughout with increasing interval between the administration of vitamin D and radiocalcium.

The Ca^{45} values of femur were closely correlated to the values of inorganic phosphorus in serum. The coefficient of correlation was 0.679, which is highly significant ($P < 0.001$). The equation of the regression line was found to be $y = 0.307 \cdot x + 1.41$ ($x =$ mg % inorganic P; $y = Ca^{45}$ uptake).

Discussion

The average specific activity of the serum calcium was higher throughout the whole time of the experiment in the untreated animals than in those treated with vitamin D (table VI). A correction of Ca^{45} values of the skeleton with regard to the specific activity of serum calcium therefore tends to increase the difference still more between these two groups. On account of the difficulties in determining the average specific activity of serum calcium in every experiment, it may be justified in the calculation of the effect of the vitamin on the skeleton to use the values uncorrected.

The radiocalcium uptake in the femur of the untreated animals was found to be 1.8 per cent of the dose given, the corresponding figure for the vitamin D treated animals being 3.0 per cent, i. e. an increase of about 65 per cent. This value is in good agreement with the results obtained by GREENBERG (1945). This increase cannot, however, be forthwith ascribed to a direct effect of vitamin D on the mineralization of the skeleton. It may be secondary to an additive effect that increased absorption of inactive calcium from the digestive tract may have on calcification, for one must take into account the possibility that the subcutaneously administered radiocalcium may readily mix with the inactive calcium coming from the digestive tract. The absorption from the digestive tract is, however, probaly only slight, because the animals had been deprived of food for 6 hours before the administration of the radiocalcium. Another possibility is that the increased uptake of radiocalcium by the skeleton in the vitamin D treated animals is

secondary to an increase in the inorganic phosphorus in the serum, which was also found in these experiments.

As mentioned before, the administration of vitamin D is followed by a decrease in the amount of radiocalcium recovered from the digestive tract + faeces (table VII). This does not necessarily mean that vitamin D affects the passage of calcium into the bowel. The difference found between the values recorded may be attributable to a difference in the re-absorption of calcium from the intestines of the animals in either group. The continuous decrease of radiocalcium in the digestive tract with increasing interval between the administration of vitamin D and radiocalcium gave a curve of the same shape as that obtained for the absorption of radiocalcium when it was given orally under the same experimental conditions (chapter V). This supports the secretion-reabsorption theory for calcium in the intestine (NICOLAYSEN 1934, 1951), a theory in opposition to the opinion that calcium is excreted through the intestinal walls (STEGGERDA & MITCHELL, 1946, FOURNIER, 1951).

Summary

In rachitic rats the skeletal uptake of parenterally administered radiocalcium was found to be greater in those treated with vitamin D than in the untreated ones. This does not, however, necessarily mean that the vitamin D has a direct effect on the mineralization of the skeleton, because it may be a secondary effect.

The radiocalcium values obtained from the digestive tract + faeces following the parenteral administration of the isotope were smaller in the vitamin D treated animals than in the untreated ones. This difference is probably due to an increased re-absorption of the calcium in the vitamin D treated rats.

CHAPTER VII

Effect of Vitamin D on the Absorption of Radiocalcium from the Digestive Tract, as Observed at Varying Intervals after the Administration of the Isotope

A point of considerable interest in the study of the effect of vitamin D on the absorption of calcium is in what section of the intestine the vitamin exerts its effect. According to earlier investigations the absorption of calcium and of phosphorus occurs exclusively in the small intestine. In experiments on isolated jejunal loops NICOLAYSEN (1937 C, 1951), also found that the rate of absorption of calcium is much lower in vitamin D deficient rats than in rats fed on the same diet but treated with vitamin D. HARRISON & HARRISON (1951), however, concluded from their experiments with radiocalcium that the effect of vitamin D on the absorption of calcium is observed only under conditions in which the calcium of the intestinal contents is poorly soluble, i. e. when the calcium is in the distal portion of the intestine. It is, however, difficult to evaluate the results of HARRISON & HARRISON, because the effect of vitamin D was much weaker than that observed in the present experiments, and their series was small. CARLSSON (1952, B) mentions an experiment in which the result obtained agrees to a certain extent with HARRISON & HARRISON'S view. As knowledge of the conditions under which vitamin D exerts its effect on the intestine is obviously of great interest, there were good reasons to elucidate this question further with tracer experiments.

The purpose of the experiments described below was to study the effect of vitamin D on the absorption of radiocalcium in rachitic rats at varying intervals after the administration of a single dose of this isotope.

Experiments

Seventy seven rachitic rats were used. Before the commencement of the experiment the animals were divided into 2 equal main groups, one of which received 15 I.U. vitamin D 4 days before the administration of radiocalcium. Vitamin D was given in the form of calciferol dissolved in 0.5 ml. peroxide-free peanut oil. The other group received 0.5 ml. peanut oil only. After the animals had been starved for 6 hours 20 mg. radiocalcium was administered as the lactate to each of the animals via a stomach tube, the total quantity of the fluid thus administered was 2 ml. The animals were then killed at varying intervals after the administration of the radiocalcium. The first, second, third and fourth groups that were killed after 1, 2, 4 and 6 hours respectively, received no food during this interval, while the other 2 groups that were killed after 12 and 18 hours respectively, received their usual diet as from 3 hours after the administration of the radiocalcium. Table IX gives the number of animals in each of the groups. The blood, digestive tract + faeces, urine and skeletal parts were collected and examined for radiocalcium. At dissection of some of the animals of the first 4 groups the stomach, the upper half of the small intestine, the lower half of the small intestine and the large intestine were removed separately. The faeces were added to the fraction of the large intestine.

Results

The specific activity of serum calcium at varying intervals after the administration of radiocalcium was described in chapter III (Fig. 4).

The other determinations are summarized in table VIII and Fig. 12. It was apparent from Fig. 12 that an effect of vitamin D on the absorption of radiocalcium could be observed already one hour after the administration of the isotope ($P < 0.01$). The difference between the 2 groups as regards the absorption of radiocalcium increased for the following hours, and the effect of vitamin D on the absorption did not reach a maximum until 4—6 hours after the administration of Ca^{45} (the increase per cent in the absorption was 38.3, 24.4, 50.9 and 64.2 after an interval of 1, 2, 4 and 6 hours respectively). The figure also shows that the absorption in the untreated animals ceased after about 2 hours, while it continued for another 2—4 hours in the vitamin D treated ones. This difference might be ascribable to

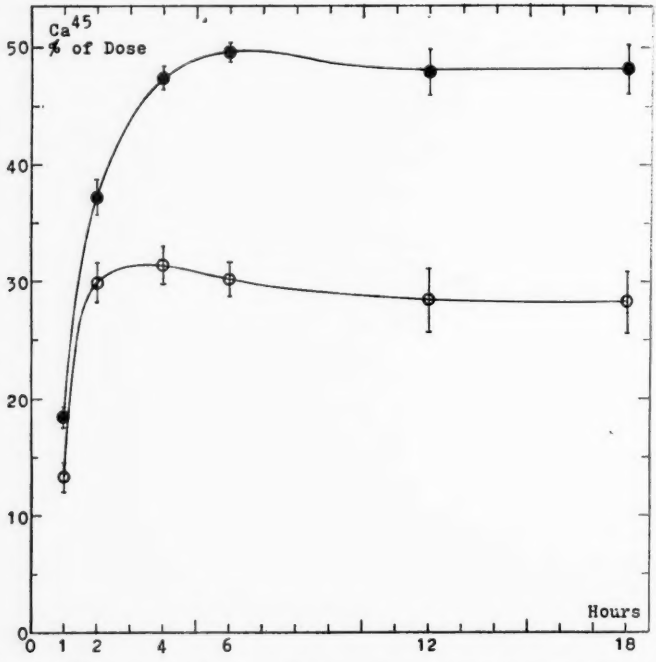

Fig. 12. Effect of vitamin D on the absorption of Ca^{45} from the digestive tract as observed at a varying interval after the administration of the isotope.
Solid symbols: Vitamin D treated
Open symbols: Untreated

a difference in the rate of the passage of the intestinal contents, the stomach being emptied slower in animals treated with vitamin D. But this was not the case, because the passage was, if anything, somewhat quicker in this animals, at least initially (table VIII), which may explain why the effect on the absorption is greater after 1 hour than after 2. After an interval of 6 hours the absorption values decreased in both groups. This decrease is probably only illusory and due to a return to the intestine of previously absorbed radiocalcium, by which the

Table VIII. *Radiocalcium recovered from different parts of the digestive tract of vitamin D treated and untreated rachitic rats as observed at a varying interval after the oral administration of the istope.*

Vitamin D	Hours after Ca^{45}	Nr. of rats	Stomach	Small intestine		Large intestine + feces
				upper part	lower part	
+	1	3	13.3	13.4	26.9	26.0
−	1	3	11.4	12.8	36.5	22.5
+	2	6	1.6	0.7	14.5	45.4
−	2	5	4.8	3.3	22.9	33.5
+	4	4		0.6	3.6	49.0
−	4	4		0.9	3.4	65.6
+	6	3			0.9	50.5
−	6	3			1.1	66.3

amount of radiocalcium recovered from the digestive tract + faeces will increase somewhat. Consequently the figures obtained for the absorption will be somewhat lower.

Table VIII shows that in the untreated animals there was still a considerably amount of radiocalcium — almost 50 per cent of that not absorbed — in the stomach and small intestine 2 hours after the administration of the isotope, i. e. when the absorption in this group had practically ceased. This quantity then passed into the large intestine without further absorption. The corresponding values for the animals treated with vitamin D was 35 per cent. But in these animals absorption continued for another 2—4 hours, by when these parts of the intestine were practically emptied of their content of radiocalcium. By this time the absorption of radiocalcium had practically ceased in this group.

Discussion

These results differ from those obtained by HARRISON & HARRISON (1951) in that absorption took place in both groups only when some radiocalcium was present in the small intestine. Thus no absorption from the large intestine was demonstrated in the animals treated with vitamin D. Consequently the results

of this investigation confirm earlier opinions on this point i. e. that only the small intestine is normally involved in the absorption of calcium.

Another deviation from HARRISON & HARRISON'S results was that an effect of the vitamin on the absorption was observed already during the first few hours after the administration of the radiocalcium. After an interval of 4 hours, when a maximum effect of the vitamin D on the absorption was observed in the present investigation, they noticed no effect at all. Whether the difference between their series and the present series really did exist or whether it was simply due to the smallness of their series, is difficult to say. Further, HARRISON & HARRISON do not state the Ca/P ratio of the diet they used.

So far no definite explanation can be given to the phenomenon that the effect of vitamin D on the Ca^{45} absorption is dependent on the interval after the administration of the isotope. It is tempting to suggest, however, that the absorption of calcium from the digestive tract occurs by means of two mechanisms: one independent of vitamin D, and another that is controlled by the vitamin.

Summary

The effect of vitamin D on the absorption of radiocalcium from the digestive tract was observed already one hour after the administration of the isotope. Not only the absolute difference but also the difference per cent in the absorption between the vitamin D treated and the untreated animals then increased for the following hours, and the effect of vitamin D on the absorption did not reach a maximum until 4—6 hours after the radiocalcium was given. An effect of vitamin D on the absorption was observable only as long as the small intestine contained some radiocalcium and from the large intestine no absorption of radiocalcium at all could be demonstrated.

The observations made suggest that the absorption of calcium from the digestive tract occurs by means of two mechanisms: one independent of vitamin D, and another that is controlled by the vitamin.

CHAPTER VIII

Effect of Vitamin D on the Skeletal Distribution of Radiocalcium in Rachitic Rats, as Observed at a Varying Interval after the Oral Administration of the Isotope

As mentioned in chapter I, MIGICOWSKY & EMSLIE (1948) expressed the view that vitamin D acts on the newly formed bone by resisting the solution effect of an adverse mineral equilibrium, which exists in rachitic animals. Rickets, they say, results because bone calcium is being dissolved and they conclude that the initial action of vitamin D is to prevent the solution of bone calcium. It was pointed out earlier (chapter I) why these results are untenable. Strong evidence is instead available that vitamin D — directly or indirectly — promotes a deposition of calcium salts in the skeleton. Results described in chapter III also suggest that, contrary to the opinion of MIGICOWSKY & EMSLIE, vitamin D promotes the passage of calcium from the skeleton into the blood.

With the aid of radioactive isotopes it has been possible to study not only the uptake of bone salts in the skeleton, but also its removal (NORRIS & KISIELESKI, 1948, CARLSSON, 1951, COPP *et al.*, 1951, ARMSTRONG & SINGER, 1951). The purpose of the experiments described below was to study the effect of vitamin D on the skeletal distribution of radiocalcium in rachitic rats at different intervals after the administration of the isotope.

Experiments

The experiments were carried out on 2 series. In both series rachitic rats received 15 I.U. vitamin D dissolved in peanut oil 4 days before the administration of the radiocalcium. They were killed at an interval varying

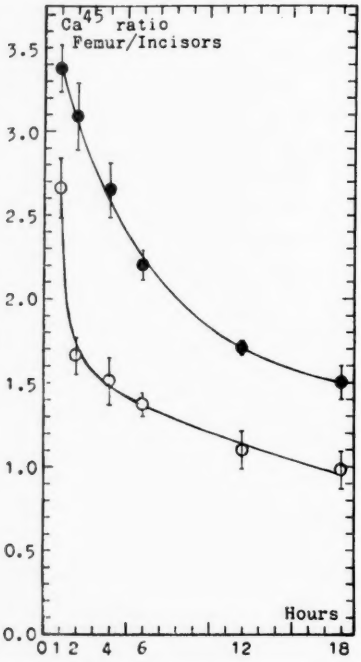

Fig. 13. Ca^{45} ratios Femur/Incisors at a varying interval of 1—18 hours after the administration of the isotope to rachitic rats vitamin D treated (solid symbols) and untreated (open symbols).

from 1 hour to 12 days afterwards. At every interval a corresponding number of control animals that had received only peanut oil were also killed. The first series, which covered an interval of 1—18 hours consisted of the same animals as those accounted for earlier in chapter VII. In the other series, in which the animals were killed 18 hours, 3, 6, 9 and 12 days after the administration of Ca^{45} some of the animals received a dose of 50 000 I.U. vitamin D under otherwise similar conditions. In order not to weaken the effect of the vitamin in the second series, a similar dose of the vitamin was administered every fourth day during the experiment.

A dose of 20 mg. radiocalcium was administered via a stomach tube in a single dose in the form of the lactate. The radioactivity of the incisors, femur and tibia-fibula was determined.

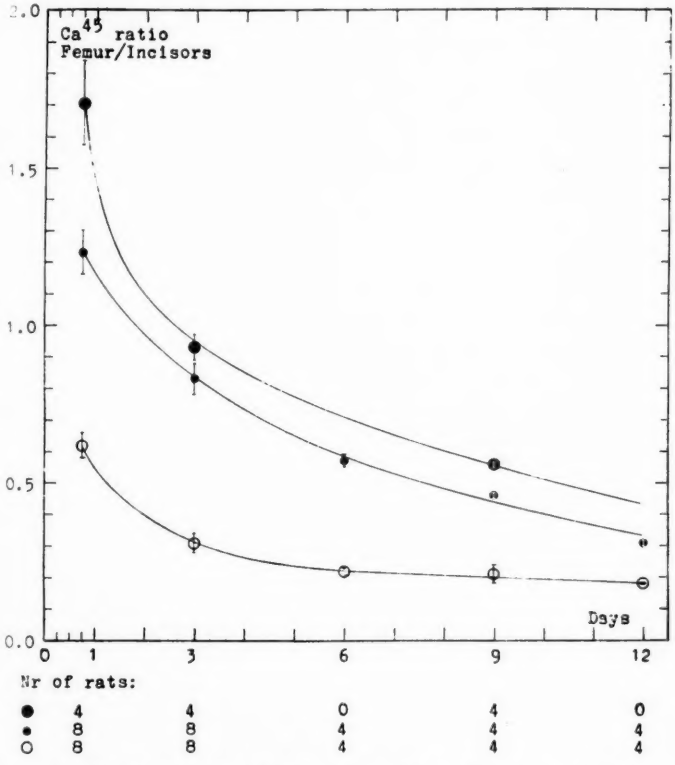

Fig. 14. Ca^{45} ratios Femur/Incisors at a varying interval of 18 hours—12 days after the administration of the isotope to vitamin D treated and untreated rachitic rats.

Large solid symbols: 50 000 I.U. vitamin D
Small solid symbols: 15 I.U. vitamin D
Open symbols: No vitamin D

Results

The results are summarized in tables IX and X and in Figs. 13 and 14. The weights of the rats were those recorded on the days the animals were killed.

The skeletal turnover of radiocalcium was assessed by com-

paring the amounts of radiocalcium recovered from the femur and from the incisors (CARLSSON, 1951). The value obtained in this way is independent of variations in the specific activity of the serum calcium.

The values obtained for the femur/incisor ratio during the first 18 hours are summarized in Fig. 13. Owing to the relatively larger uptake of radiocalcium by the femur in the vitamin D treated animals, this ratio was larger throughout in this group. The curve for both treated and untreated animals fell rapidly. Fig. 14 gives data about the Ca^{45} ratio femur/ incisor at intervals of more than 18 hours. The course of the functions for the 2 groups treated with vitamin D are similar. Owing to the larger uptake of radiocalcium in the femur of the animals that received a dose of 50 000 I.U. than of those receiving 15 I.U. (see chapter III) the Ca^{45} ratio for the firstmentioned group was somewhat higher throughout. As before the functions of the vitamin D treated animals showed a sharp fall for the 18 hours—3 days interval, after which the slope became less pronounced. But between the third day and the twelfth day there was still an obvious decrease in the femur incisor ratio. The Ca^{45} ratio for the untreated rachitic rats also decreased considerably between the 18 hours—3 days interval, after which the curve pursued a practically horizontal course.

Tables IX and X show the ash weight and the radiocalcium values for different parts of the skeleton. The ash weight of all parts of the skeleton examined were found to increase with increasing interval, and this increase was, of course, more pronounced among the animals treated wiht vitamin D. But this cannot explain the difference in the course of the Ca^{45} ratio femur/incisors between the vitamin D treated and untreated animals, which was most readily observed at intervals of 3 days and more. An increase in the ash weight for the former group means an increased calcification in these animals whereby part of the radiocalcium removed from the skeleton is taken up again together with other calcium by the skeleton. This, of course, decreases the drop in the Ca^{45} ratio femur incisor and thus tends to flatten the curve. As described earlier

Table IX. *Effect of vitamin D on the ash weight and skeletal distribution of radiocalcium in rachitic rats at intervals of 1—18 hours after the administration of the isotope.*

Time after Ca^{45}	Vitamin D	Nr. of rats	Body wt grams	Ash mg				Ca^{45} % of dose			
				Femur	Incisors	Tibia-fibula ends	Tibia-fibula shafts	Femur	Incisors	Tibia-fibula ends	Tibia-fibula shafts
1 hr	+	9	76.2	34.8	97.3	44.8	36.6	0.52	0.16	0.85	0.13
»	—	8	76.6	31.4	94.1	36.5	35.6	0.32	0.12	0.46	0.10
2 hr	+	7	71.3	31.4	96.9	41.0	33.0	1.09	0.35	1.90	0.27
»	—	7	71.4	27.4	94.1	30.0	31.6	0.53	0.32	0.70	0.21
4 hr	+	7	70.3	30.9	94.6	39.6	32.3	1.58	0.60	2.60	0.38
»	—	6	70.0	29.3	92.0	32.5	32.7	0.76	0.50	1.10	0.30
6 hr	+	8	80.1	39.8	100.1	49.4	40.4	1.59	0.72	2.56	0.43
»	—	8	79.8	33.5	98.1	36.5	38.0	0.83	0.61	1.22	0.35
12 hr	+	5	82.8	40.6	103.2	50.0	42.4	1.45	0.85	2.40	0.44
»	—	4	89.0	39.9	100.8	44.7	44.0	0.69	0.63	1.03	0.29
18 hr	+	4	85.8	40.1	104.3	53.2	43.5	1.48	0.99	2.50	0.47
»	—	4	85.5	39.0	101.5	44.7	46.0	0.72	0.74	1.01	0.33

Table X. *Effect of vitamin D on the ash weight and skeletal distribution of radiocalcium in rachitic rats at intervals of 18 hours—12 days after the administration of the isotope.*

Time after Ca^{45}	I. U. vitamin D	Nr. of rats	Body wt grams	Ash mg				Ca^{45} % of dose			
				Femur	Incisors	Tibia-fibula ends	Tibia-fibula shafts	Femur	Incisors	Tibia-fibula ends	Tibia-fibula shafts
18 hr	50.000	4	67.5	32.4	99.8	44.3	32.0	1.62	0.96	2.88	0.41
»	15	8	78.9	30.9	105.6	40.9	36.5	1.35	1.11	2.33	0.45
»	—	8	73.3	28.9	98.0	31.0	36.4	0.54	0.86	0.78	0.34
3 d	50.000	4	67.5	34.9	106.3	47.0	28.8	1.44	1.56	2.25	0.46
»	15	8	70.1	38.1	107.4	48.8	36.6	1.25	1.53	2.02	0.48
»	—	8	75.5	30.1	103.1	32.3	36.0	0.40	1.25	0.57	0.27
6 d	15	4	79.0	41.3	115.0	51.5	34.3	0.96	1.69	1.31	0.40
»	—	4	76.0	32.7	109.5	34.5	39.8	0.27	1.25	0.31	0.19
9 d	50.000	4	64.5	39.5	111.8	45.0	31.5	1.06	1.91	1.31	0.53
»	15	4	71.0	35.8	115.8	42.3	31.5	0.93	2.03	1.21	0.51
»	—	4	75.5	30.5	110.3	37.5	35.5	0.33	1.56	0.44	0.29
12 d	15	4	86.0	46.5	130.0	53.3	40.0	0.70	2.27	0.78	0.44
»	—	4	94.0	30.1	116.3	33.0	39.0	0.23	1.31	0.24	0.18

in connection with the distribution of radiocalcium in normal animals (CARLSSON 1951), the radiocalcium values for the incisors showed a continuous rise in activity throughout the experiments, which was in accord with the opinion advanced by HEVESY, LEVY & REBBE (1940) that the calcified tissue in the teeth is formed mainly in the apical part and then pushed forwards in toto experiencing only a restricted atomic or molecular replacement. Later, CARLSSON (1952 A), who compared the amount of Ca^{45} in the serum with that in the incisors during the first 18 hours after the administration of radiocalcium, found that the uptake of calcium in the incisors of the rat is mainly an irreversible process. Table X also shows that the radioactivity of the long bones, especially their ends, gradually decreased with increasing intervals. The vitamin D treated animals differed from the untreated ones in so far as the radiocalcium values for the incisors seemed to show a more marked rise, and the radiocalcium samples for the long bones tended to show a larger loss of activity.

Discussion

COPP *et al.* (1951) studied the turnover of radiocalcium in rachitic rats at varying intervals after the intravenous injection of practically carrier-free radiocalcium. They used a diet with an extremely low phosphorus content (0.008—0.015 per cent) and containing so much vitamin D that the animals received about 40—50 I.U. daily. After a maximum uptake in the skeleton 1 hour after the administration of radiocalcium there was a rapid loss of radiocalcium, so that the percentage of the dose retained by the skeleton had decreased by about 50 per cent in one hour. The slope of the curve then gradually decreased, and about 4 days after the administration of the radiocalcium the curve fell only slightly. According to these authors, the first stage suggests the presence of a very labile calcium fraction in the bones, and they suppose that this fraction of the uptake of radiocalcium is ascribable to exchangeable calcium on the surface of the bone crystals. The individual values

recorded for the radiocalcium uptake in the femur in the present investigation did not show such a rapid fall as those observed by COPP *et al.* This can probably be explained by the fact that they used a diet extremely low in phosphorus, with a resultant increased urinary excretion of calcium. A comparison between the distribution of radiocalcium in these experiments and in those of COPP *et al.* as regards the metabolism of calicum in rickets is difficult, because the rats they used in their studies were not vitamin D deficient. Neither could their animals be considered as being in the healing stage, because no signs of skeletal healing were observed despite the abundant administration of vitamin D, probably due to the low phosphorus content of the diet.

As pointed out before, the Ca^{45} ratio femur/incisors showed a rapid fall in both groups. No definite explanation can at present be offered for this drop. The possibility of surface exchange cannot be excluded, although no evidence is available to suggest that such exchange plays an important part in this connection *in vivo*. It should also be mentioned that the formation and destruction of calcified tissue may occur much more rapidly than often hitherto assumed. The initial drop seems to be most pronounced in the untreated animals (Fig. 13), which would mean that surface exchange, if any, would play a relatively greater part in these animals than in those treated with vitamin D. This would in turn decrease the difference in the uptake of radiocalcium by that part which is ascribed to a deposition of calcium salts. In chapter III evidence was presented suggesting that calcium was removed from the bones to the blood quicker in the vitamin D treated animals than in the untreated ones. This quicker removal can probably not be ascribed to surface exchange, because such exchange should be larger in the rachitic rats, but is reasonably due to an increased resorption of calcium salts from the bones. The fact that the slope of the radiocalcium curve was much greater for the vitamin D treated animals than for the others after an interval of more than 3 days, by when the effect of surface exchange could practically be excluded, also argues for this supposition. This is

also in line with the results DODDS & CAMERON (1938) obtained with their histologic investigation of the healing process in the head of the tibia in rachitic rats. They found that before the calcified band in the metaphysis had become very wide, the removal of the calcified osteoid began close to the cartilage and the removal process advanced steadily toward the shafts. They also emphasized that there is reason to believe that the very earliest calcification occurs in the masses of osteoid, which are very early removed in the first enlargement of marrow spaces close to the edge of the cartilage.

Another question is to what extent the dissolution effect of vitamin D in rickets is primary or secondary and due to the presence of osteoid that is believed to inhibit the removal of calcified tissue (WEINMANN & SICHER 1947, BAILIE & IRVING 1948). In this respect the observations reported by CARLSSON (1952 C) are of interest. In experiments on rats fed on a diet extremely low in Ca, evidence was presented that vitamin D promoted the removal of Ca from the bones and that under these conditions the increase in serum calcium level and the increased growth and calcification of the skeleton were secondary to this effect. It therefore seems probable that the promoting effect of vitamin D on bone resorption in healing of rickets is ascribable to a direct influence on the disintegration and removal of calcified tissue.

The shape of the two curves (Fig. 14) for the vitamin D treated rats thus seems to be interpretable as a sign that a physiologic dose and a massive dose have principally the same effect on bone resorption. This means that the decalcification of the bones following the administration of massive doses of vitamin D at least partly is an accentuation of the physiologic effect. In explaining the effect of massive doses of vitamin D on the skeleton it therefore does not seem necessary to assume the occurrence of toxic decomposition products in the body as a cause of this effect (REED 1950).

Summary

The effect of vitamin D on the skeletal distribution of Ca^{45} at different intervals after the administration of radiocalcium was studied. Both in vitamin D treated and in untreated rachitic rats the Ca^{45} ratio femur/incisors decreased markedly during the first 18 hours after the administration of the isotope. The slope of the Ca^{45} ratio curve then gradually decreased, but more in the untreated animals than in the treated ones. This difference was most readily demonstrable after an interval of more than 3 days. The data agree with the assumption that by healing of rickets in rats after administration of vitamin D, there is an increased process of bone resorption as compared with untreated rats.

The Ca^{45} ratio curve obtained by administration of massive doses of vitamin D showed a course parallel with the corresponding function obtained by administration of physiologic doses. Consequently it seems reasonable to assume that the effect of vitamin D in massive doses on the skeleton — decalcification of the bones — is at least partly an accentuation of the physiologic effect.

CHAPTER IX

Effect of the Addition of Phosphate to a Rachitogenic Diet on the Absorption and Skeletal Distribution of Radiocalcium

As mentioned earlier, vitamin D promotes the absorption of calcium from the digestive tract. The mechanism responsible for this effect is unknown; it may possibly be the result of a direct action of the vitamin on the intestinal mucosa, or it may be of secondary nature. MIGICOWSKY & EMSLIE (1948) believe the effect of vitamin D on the absorption to be an indirect consequence of the prevention of the entrance of bone calcium into the metabolic pool by the vitamin. HEYMAN (1937) hold it possible for vitamin D to exert a direct action on the epithelial cells in the intestinal tract. NICOLAYSEN (1951) is of the opinion that we do not yet know whether the effect is direct or indirect.

The increased absorption of calcium from the digestive tract may, however, be due to a greater requirement of calcium by the increased calcification following the administration of vitamin D. As known, mineralization of rachitic bone can be brough about not only by medication with vitamin D, but also by the correction of the Ca:P ratio to one more favourable for calcification. While vitamin D requires about 3 days to reach its full effect (see chapter V), calcification occurs much quicker after correction of the Ca:P ratio. KRAMER *et al.* (1931 A) and IRVING (1946) thus found that new calcification appeared in rachitic bones in 12—24 hours after adding phosphate to the diet. The purpose of the experiment described below was to study the absorption and skeletal distribution of radiocalcium after an addition of phosphate to the diet.

Experiments

The series was divided into 3 groups. One group received a dietetic addition of phosphate 24 hours before the administration of radiocalcium: 4.1 gm. NaH_2PO_4, 2 H_2O was added for every 100 gm. of the rachitogenic diet. This changed the Ca:P ratio from 5.9 to 1.1. These animals, like those in the subsequent groups, received no food for 6 hours before the treatment. Another group received 15 I.U. calciferol 24 hours before the administration of Ca^{45}. A third group — control group — received no treatment. Each group consisted of 8 animals. As regards litter, sex and body weight the groups were as uniform as possible.

A dose of 20 mg. radiocalcium was administered as the lactate by the stomach tube. The volume of the fluid administered was 2 ml. The animals were killed 18 hours afterwards.

Results

KARELITZ & SHOHL (1927 B) and KRAMER *et al.* (1931 A) found that the addition of P to a rachitogenic diet with a high Ca:P ratio rendered rats hyperexitable and liable to attacks of tetany. As in IRVING'S (1946) experiments, no such effect was observed in the present series. This difference may be explained by the fact that here the Ca:P ratio was not completely corrected, the amount of calcium being relatively larger even after the addition of P.

The results of the activity determinations made in this experiment are summarized in table XI.

In the group that received an addition of phosphate in the diet the uptake of radiocalcium in the femur as well as in the ends of the tibia-fibula was pronounced ($P<0.001$). Compared with the control animals, the skeletal uptake of radiocalcium in the vitamin D treated animals was also increased, ($P<0.001$), but the values recorded were not so high as in the phosphate treated group. As described earlier (see chapter V), vitamin D increased the absorption of radiocalcium already at an interval of 24 hours ($P<0.001$). No difference was, however, found between the animals treated with phosphate and the controls as regards the absorption of radiocalcium.

The specific activity of the serum calcium at the end of the

Table XI. *Effect of addition of phosphate to a rachitogenic diet on the absorption and skeletal distribution of orally administered radiocalcium.*

Vitamin D	Addition of P	Nr. of rats	Body wt grams	Ca^{45} % of dose						Serum		
				Absorption	Urine	Femur	Incisors	Tibia-fibula ends	Tibia-fibula shafts	Ca^{45} % dose per mg Ca	mg % Ca	mg % P
+	−	8	69.6	38.5 ±0.9	10.7 ±1.1	0.78 ±0.04	1.04 ±0.03	1.20 ±0.02	0.36 ±0.02	0.30	10.8	3.1
−	+	8	71.0	27.3 ±2.2	1.4 ±0.2	1.02 ±0.06	0.62 ±0.04	1.62 ±0.05	0.32 ±0.01	0.40	6.0	7.0
−	−	8	70.5	26.4 ±1.2	8.5 ±0.8	0.46 ±0.04	0.75 ±0.03	0.64 ±0.06	0.26 ±0.01	0.29	10.5	2.5

experiment, i. e. 18 hours after the administration of the radiocalcium, was the same in the vitamin D treated animals as in the controls. The corresponding value for the animals treated with phosphate, however, was somewhat higher. In this connection it may be convenient to discuss the uptake of radiocalcium in the incisors. As these teeth are affected but little by the rachitic disease (chapter V), the uptake of Ca^{45} in them can be regarded as a relative measure of the average specific activity of the serum calcium throughout the experiment. In the group that received phosphate the Ca^{45} uptake of the incisors was found to be smaller than in the control group, which definitely argues against the assumption that the increased skeletal uptake of Ca^{45} of the phosphate treated animals is secondary to an increase in the specific activity of the serum calcium. In this connection it may be of interest to note that in histological studies IRVING (1946) observed an increase in the calcification of the incisors after a similar change in the Ca:P ratio of the diet.

As shown earlier by KRAMER *et. al.* (1931 A), for example, the correction of the Ca:P ratio of the diet quickly increased the amount of inorganic phosphorus in the serum and produced a simultaneous decrease in the serum calcium.

Discussion

The administration of vitamin D was followed by an increase in skeletal calcification as well as in the absorption of Ca^{45} from the digestive tract. The increased skeletal uptake of Ca^{45} obtained by addition of phosphate to the diet — of an order slightly greater than that by the administration of vitamin D — was, however, not accompanied by an increase in the absorption of radiocalcium. It is therefore probable that the favourable effect of vitamin D on the absorption of calcium from the intestines is not secondary to an increased deposition of bone salts in the skeleton.

The increased Ca^{45} uptake in the skeleton on addition of phosphate to the diet was compensated by a considerably decreased excretion of Ca^{45} with the urine. This confirms what KARELITZ & SHOHL (1927 A, B) found in their investigation of the calcium balance. They observed that a change in the Ca:P ratio from 4.25 to 0.95 decreased the calcium in the urine both in actual amount and relatively to intake.

According to ZETTERSTRÖM (1951), the low concentration of inorganic phosphorus in serum usually found in rickets is only an additional factor depressing the ability of the osteoid tissue to calcify. However, after administration of abundant vitamin D skeletal changes will heal only if the phosphorus content of the diet is not too low (COLEMAN *et al.* 1950). In their tracer experiments on the effect of vitamin D on the healing of rickets MORGAREIDGE & MANLY (1939) found that an effect on the uptake of P^{32} by the metaphyses is not observable until 54—72 hours after the administration of the vitamin, and they take this as an indication that vitamin D exerts a direct influence on the metabolism controlling calcification. However, as pointed out by EICHLER (1951), this interpretation is not conclusive, because the authors did not take into consideration the effect of an increase of inorganic P in the serum. The increased deposition of calcium salts in the bones, which was observed on addition of phosphate to the diet is, however, very probably secondary to the increase of the inorganic phosphorus in the

serum, which, like the increased calcification, was observed very soon after the change of the diet. This suggests that the increased uptake of radiocalcium in the bones after medication with vitamin D and obtained after oral as well as after parenteral administration of Ca^{45} is also due to an appreciable extent to the simultaneous increase in the serum phosphorus, to which it was highly correlated (see chapter V and VI). Judging by recent investigations (HIATT & SHORR 1951, GUTMAN & YÜ 1951, NEUMANN, DISTEFANO & MULRYAN 1951) it is highly questionable whether any local increase in the organic P concentration occurs in the enchondral calcification; the role of the glycogenolytic cycle and the alkaline phosphatase in the process of calcification must still be regarded as unclear. The above mentioned, indirect effect of vitamin D on bone deposition is compatible with the observations by KRAMER, SHEAR & SIEGEL (1931 A), who in healing rickets found the apperance of new calcification, as observed histologically, to be preceded by an increase of the Ca x P product in the serum.

Summary

The effect of an addition of phosphate to a rachitogenic diet on the metabolism of radiocalcium was studied. Calcification induced in this way and of a degree slightly greater than that obtained by administration of vitamin D was not accompanied by an increase in the absorption of radiocalcium from the digestive tract. It is therefore probable that the favourable effect of vitamin D on the absorption of radiocalcium is not secondary to an increased deposition of calcium salts in the skeleton.

The increased deposition of bone salt observed in healing rickets is probably due to an appreciable extent to an increase of the inorganic phosphorus in the serum.

CHAPTER X

Effect of Parenteral Administration of Phosphate on the Metabolism of Radiocalcium in Rachitic Rats

As mentioned earlier, the induction of rickets in rats requires not only vitamin D deficiency but also an unfavourable Ca:P ratio in the diet, in which the important factor is lack of phosphorus. In the healing of rickets by correcting the Ca:P ratio to optimal value, the calcium absorption from the intestines was not increased in spite of the intensive calcification following such correction (chapter IX). However, under these conditions calcium and phosphorus are presented in optimal ratio. If phosphorus is given without this ratio being maintained, such as by parenteral administration of phosphates, the organism has the possibility to make up for it by increasing excretion, mainly with the urine, of the surplus (LOGAN 1940). It would, however, be of interest to know whether such a surplus may be controlled by an increased absorption of calcium.

The purpose of the experiments described below was to investigate the effect of parenteral administration of phosphate on the metabolism of radiocalcium in rachitic rats.

Experiments

The experiments were carried out on 2 series. Rickets had been induced by means of a diet high in calcium and low in phosphorus. In the first series the animals were divided into 4 groups. One group — a control group — received no treatment. The second group received 15 I.U. calciferol via a stomach tube 24 hours before the commencement of the experiment. The third group received subcutaneously 5 mg. P as the phosphate (1 ml.) simultaneously with the Ca^{45} and another 5 mg. in the same manner 1 hour

Table XII. *Effect of subcutaneous injection of phosphate on the absorption and skeletal distribution of orally administered radiocalcium.*

Vitamin D	Injection of P	Nr. of rats	Body wt grams	Ca^{45} % of dose						Serum		
				Absorption	Urine	Femur	Incisors	Tibia-fibula ends	Tibia-fibula shafts	Ca^{45} % dose per mg Ca	mg % Ca	mg % P
–	–	11	73	26.8 ±0.9	8.7 ±0.6	0.49 ±0.03	0.78 ±0.03	0.68 ±0.05	0.27 ±0.01	0.27	10.8	2.6
+	–	11	73	40.4 ±1.3	11.1 ±0.8	0.84 ±0.06	1.05 ±0.04	1.30 ±0.09	0.38 ±0.02	0.29	11.2	3.0
–	+	11	74	35.3 ±1.2	0.9 ±0.2	1.27 ±0.06	0.82 ±0.03	1.87 ±0.07	0.42 ±0.04	0.40	9.4	3.8
+	+	8	71	47.9 ±1.9	1.0 ±0.3	1.73 ±0.10	1.06 ±0.07	2.50 ±0.16	0.56 ±0.04	0.34	10.4	–

later. The fourth group received treatment with vitamin D as well as with phosphate given subcutaneously in the same manner as in groups 2 and 3. The animals were given 20 mg. Ca^{45} as the lactate by the oral route and were killed 18 hours later.

In series II the animals were treated with phosphate injections in the same manner as were group 3 in series I. The animals were then killed at intervals varying from ½—6 hours after the administration of 20 mg. Ca as the lactate. Each group consisted of 1—3 rats. Determinations of serum calcium and inorganic phosphorus were carried out.

Results and Discussion

For a few hours after the injections of phosphate all of the animals had diarrhoea, as is often the case after peroral administration of large quantities of phosphate (KARELITZ & SHOHL, 1927 A). The most striking observation was, however, the change in the general behaviour of the animals. They looked ill, their legs became stiff, they had convulsions, i. e. they looked as if they had tetany, which practically always occurs after parenteral administration of phosphate (see EICHLER, 1950). This tetany is ascribed to the reduction in serum calcium (table XIII). The serum phosphorus reached values of more than 12 mg/100 ml. during the first few hours after such an injection. But the value soon fell, and at the end of the experi-

Table XIII. *Effect of subcutaneous injection of phosphate on the values of serum Ca and inorganic P, as observed at a varying interval after oral administration of calcium.*

	Interval after administration of Ca						
Hours	1/2	1 1/2	2	2 1/2	3	4 1/2	6
Mg Ca/100 ml	10.0	8.5	7.1	5.8	5.8	5.2	5.3
Mg P/100 ml	7.1	10.5	12.9	9.2	10.0	8.8	7.5

ment, i. e. 18 hours after the administration of Ca^{45}, it was 3.8 mg/100 ml. By this time the serum calcium has also reached normal values.

Parenteral administration of phosphate to rachitic rats that were simultaneously given Ca^{45} *per os* produced a pronounced uptake of Ca^{45} by the skeleton (table XII). In the same group the excretion of radiocalcium with the urine was very low. In the control animals that received no phosphate, the skeletal uptake of Ca^{45} was much smaller. The immediate effect of the injection of phosphate on the Ca^{45} uptake by the bones cannot be accounted for any other process than an increased deposition of calcium salts.

The control animals and the animals treated only with vitamin D showed the same values regarding absorption and skeletal distribution of Ca^{45} as described in previous chapters. In the animals that had received injections of phosphate the absorption of Ca^{45} increased. It was not increased to a high degree — the increase was smaller than that following vitamin D administered 24 hours before the experiment — but it was statistically significant ($P < 0.001$). In the animals treated with both vitamin D and phosphate the absorption of radiocalcium from the intestines was larger than in the animals treated with vitamin D only. As was the case in the animals that received phosphate only the Ca^{45} excretion with the urine was low. The effect of injection of phosphate on the retention of radiocalcium can be studied by the following formula (Ret = retention):

$$Ret_{P-inj.} - Ret_{control} = Ret_{P-inj.+vit.D} - Ret_{vit.D}$$

The value on the left of the equation was 16.3 and that on the right was 17.6. This suggests that the effect of the injection of phosphate on the absoption of Ca^{45} from the intestine is independent of the effect of vitamin D.

The pronounced fall in the serum Ca under these experimental conditions is noticeable (cf. McLean & McCoy, 1936). In this connection it should be pointed out that parenteral administration of Ca lactate to normal and Ca deficient rats increased the uptake of Ca^{45} from the gastrointestinal tract, probably due to an inhibition of gastrointestinal movements (Carlsson, 1951). But the explanation can hardly be the same in the experiments described above, because the animals instead showed signs of increased intestinal movements. According to Nicolaysen (1937 C) the absorption of calcium from the intestines does not differ demonstrably from substances which pass through the intestinal wall by means of simple diffusion. He found that the absolute uptake of Ca from isolated intestinal loops was to a high degree dependent on the concentration of the solution administered. Obviously the absorption must also be related to the concentration of the substance in question on the other side of the membrane, i. e. in the tissue fluid. This concentration must, however, be very low if it is to accelerate absorption, because no increase in absorption was found on correction of the Ca:P ratio with a following decrease in the serum Ca to about 6 mg./100 ml. (chapter IX). The increased absorption of Ca^{45} obtained in the present experiment by injections of phosphate can therefore hardly be explained by a decrease of the serum calcium level, especially as this had not fallen so much until 6 hours after the administration of calcium, when the absorption had practically ceased (chapter VII). It cannot, of course, be excluded that a very pronounced drop in the serum Ca can give rise to a certain increase in the Ca-absorption by increased diffusion, but this shift would undoubtedly have to be so large that it can hardly be expected to occur in physiologic conditions.

Summary

Parenteral administration of phosphate to rachitic rats that were simultaneously given radiocalcium *per os* gave a prompt and pronounced uptake of Ca^{45} by the bones. This cannot be accounted for by any other process than an increased deposition of calcium salts.

Parenteral administration of phosphate to rachitic rats increased the absorption of radiocalcium given orally in the form of the lactate and simultaneously with the phosphate. This effect, which was also observed in animals that had received vitamin D, is probably of secondary nature.

CHAPTER XI

General Summary

The purpose of the present study was to investigate the effect of vitamin D on the absorption, excretion and skeletal metabolism of radiocalcium in rachitic rats.

Rickets was produced experimentally in rats by feeding them for 3 weeks on a modified Steenbock rachitogenic diet Nr. 2965. Some of the animals then received a single dose of calciferol dissolved in peanut oil, while their litter mates received peanut oil only. At varying intervals after this administration the animals received radiocalcium in the form of a single dose, either 20 mg. by stomach tube or 10 mg. subcutaneously. The animals were usually killed about 18 hours after the introduction of the isotope. Activity determinations were made of the blood, excreta and different parts of the skeleton. The results may be summarized as follows.

Absorption of Ca^{45}

The absorption of radiocalcium from the digestive tract increased with increasing dose of vitamin D up to a certain level, beyond which further increase of the dose failed appreciably to increase the absorption. Under the conditions used in the present investigation this dose was about 15 I.U. Increasing the dose to 50 000 I.U. did, however, produce a slight, further increase in the absorption.

It was also shown that the effect of a physiologic dose of vitamin D on the absorption of Ca^{45} was demonstrable already at an interval of 4—12 hours between the administration of vitamin D and radiocalcium. The effect then gradually in-

creased and reached a maximum at an interval of 60—72 hours. At an interval of 9 days the effect began to diminish, and then to pass off after altogether about 16 days. These observations show that vitamin D has a gradually increasing effect, demonstrable after a fairly short time.

It was noted that the absorption of radiocalcium after a single dose of vitamin D to rachitic rats — as observed 4 days after the vitamin was given — was practically the same as after the prophylactic administration of the vitamin.

The radiocalcium values obtained from the digestive tract + faeces following the parenteral administration of the isotope were smaller in the vitamin D treated animals than in the untreated ones. This does not necessarily mean that vitamin D affects the passage of calcium into the bowel. The difference between the values recorded is probably due to an increased re-absorption of calcium in the vitamin D treated rats.

An effect of vitamin D on the absorption of radiocalcium from the digestive tract can be observed already one hour after the administration of the isotope. Not only the absolute difference but also the difference per cent in absorption between the vitamin D treated and the untreated animals then increased during the following hours, and the effect of vitamin D on the absorption did not reach a maximum until 4—6 hours after the radiocalcium had been given. An effect of vitamin D on the absorption was observable only as long as the small intestine contained some radiocalcium, and from the large intestine no absorption of radiocalcium at all could be demonstrated. These observations suggest that the absorption of calcium from the digestive tract occurs by means of two mechanisms: one independent of vitamin D, and another that is controlled by the vitamin.

The effect of an addition of phosphate to a rachitogenic diet on the metabolism of radiocalcium was also studied. The increase thus obtained in the skeletal uptake of Ca^{45} — of an order slightly greater than that by administration of vitamin D — was not accompanied by an increase in the absorption of radiocalcium from the digestive tract. It is therefore probable

that the favourable effect of vitamin D on the absorption of calcium is not secondary to an increased deposition of calcium salts in the skeleton.

Parenteral administration of phosphate to rachitic rats increased the absorption of radiocalcium which was simultaneously given *per os*. This effect, seen also in animals treated with vitamin D, is probably of secondary nature.

Metabolism of Ca^{45} in calcified tissue

Investigation of the specific activity of serum calcium in vitamin D treated and untreated rachitic rats suggests that an increased uptake of Ca^{45} in the bones on administration of vitamin D represents an increased deposition of calcium salts. This is also supported by the observation that parenteral administration of phosphate to rachitic rats that were simultaneously given radiocalcium *per os* was followed by a quick and marked uptake of Ca^{45} in the skeleton and by a simultaneous pronounced drop in the level of the urinary Ca^{45}, while the uptake of Ca^{45} in a corresponding group receiving no phosphate was much smaller.

It was shown that the response of rachitic rats to the administration of radiocalcium is quantitatively related to the dose of vitamin D given and that the curve for the uptake of Ca^{45} in the bones has the shape of a logarithmic function. A biological assay for vitamin D was devised on the basis of this observation. According to this assay, the response to a given dose of vitamin D is recorded in the form of measurements of the radioactivity of the ends of the tibia-fibula 18 hours after the administration of the isotope.

When vitamin D was given at different intervals before the administration of radiocalcium the curve for the uptake of Ca^{45} in the bones showed practically the same course as that described above for the absorption. The incisors, however, were found to take up the maximum percentage of a given dose of Ca^{45} when the vitamin was administered already 24—48 hours

before Ca^{45}, probably on account of the fact that the mineralization of the incisors is less effected than the rest of the skeleton in rickets.

In rachitic rats the skeletal uptake of parenterally administered radiocalcium was found to be greater in those treated with vitamin D than in the untreated ones. This does not permit the conclusion that vitamin D exerts a direct effect on the mineralization of the skeleton, because this effect may be secondary. The increased deposition of calcium salts in the bones which was observed on addition on phosphate to the diet, is, however, very probably secondary to the increase of the inorganic phosphorus in the serum, which — like the increased calcification — was observed very soon after the change of the diet. This suggests that the increased uptake of radiocalcium in the bones after medication with vitamin D and obtained after oral as well as after parenteral administration of Ca^{45}, is also due to an appreciable extent to the simultaneous increase of inorganic phosphorus in the serum. The correlation between the inorganic phosphorus in the serum and the uptake of radiocalcium in the femur was statistically highly significant.

The administration of vitamin D did not increase the specific activity of the serum calcium despite an increase in the amount of radiocalcium entering the blood from the intestines. The most reasonable explanation available for this phenomenon is that vitamin D simultaneously increases the amounts of inactive calcium entering the blood stream from the bones. The problem of dissolution of bone salts was also studied by comparing the amounts of radiocalcium recovered from the femur and from the incisors at different intervals after the administration of the isotope. Both in vitamin D treated and in untreated rachitic rats the Ca^{45} ratio femur/incisors decreased markedly during the first 18 hours after the administration of the isotope. The slope of the Ca^{45} ratio curve then gradually decreased, but more in the untreated animals than in the treated ones. This difference was most readily demonstrable after an interval of more than 3 days. The data

agree with the assumption that in healing rickets in rats after administration of vitamin D, there is an increased removal of bone salt as compared with untreated rats.

The Ca^{45} ratio curve obtained by administration of massive doses of vitamin D showed a course practically parallel with the corresponding function obtained by administration of physiologic doses. Consequently it seems reasonable to assume that the effect of vitamin D in massive doses on the skeleton — decalcification of the bones — is at least partly an accentuation of the physiologic effect.

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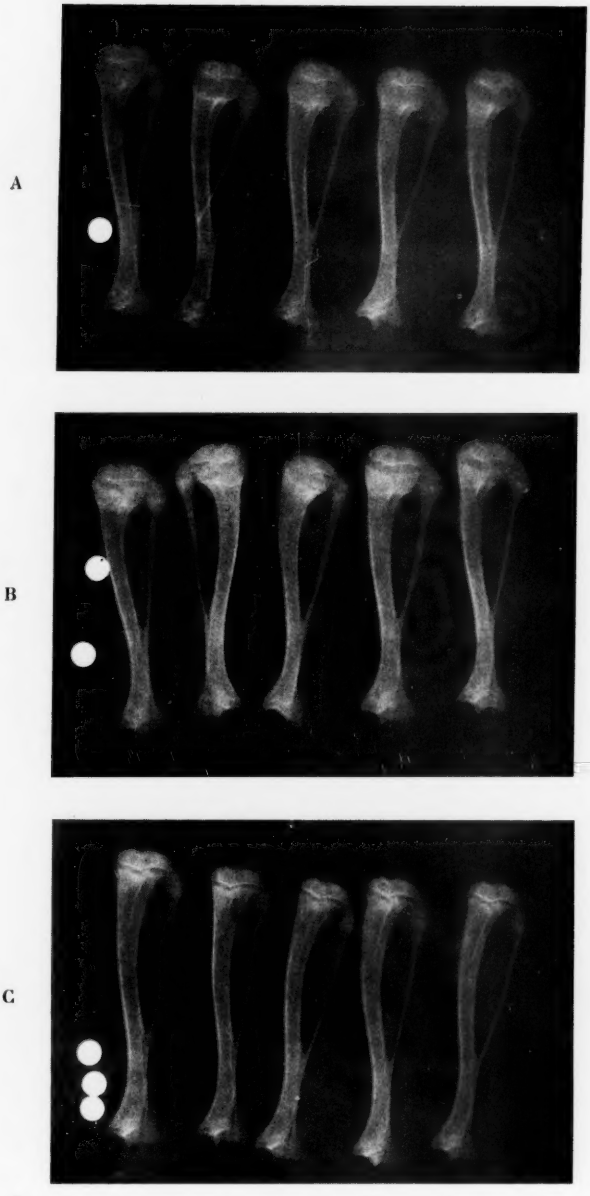

Fig. 6. Roentgenogram of the tibia-fibula of rats in groups A, B and C summarized in table IV.

FROM THE PAEDIATRIC CLINIC OF THE UNIVERSITY HOSPITAL, UPPSALA

STUDIES ON BLOOD ZINC

A Clinical and Experimental Investigation into the Zinc Content of Plasma and Blood Corpuscles with Special Reference to Infancy

By

RAGNAR BERFENSTAM

UPPSALA 1952
ALMQVIST & WIKSELLS BOKTRYCKERI AB

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CHAPTER I

Introduction

Although zinc has long been a familiar ingredient in medicaments for external use, it was for a long time not known to have any biological significance, and therefore did not prompt much study into its presence in the organism. At the end of the last and beginning of this century, however, a number of investigations were made[1], into the occurrence of zinc in biological material. It is difficult to assess the value of these investigations, however, since judged by present standard several of the methods used cannot be considered satisfactory.

Many of the investigations into the zinc content of blood and organs undertaken during the 1920's and '30's were made in the interests of an occupational disease called *metal fume fever*, attributed to the inhaling of zinc fumes, and affecting workers in zinc foundries. The symptoms are high temperature, with chills, headache, and general malaise, and the disease is thought to arise from a pyrogenous combination of zinc with the mucosal protein of the respiratory tract (LEHMANN 1910). Improved ventilation in the workshops has now lessened the frequency of the disease. The blood zinc studies made in connection with *metal fume fever* are, unfortunately, open to the same objections as those mentioned above.

Earlier on, it was generally assumed that *insulin* always contained small quantities of zinc. This has subsequently been questioned, however, and SANGER's investigation of 1949 proved that the physiological activity of insulin is not dependent on

[1] For details *cf.* The biological, hygienic, and medical properties of zinc and zinc compounds, Public Health Report Suppl. no 179, Washington 1945 and VALLEE & ALTSCHULE: Zinc in the mammalian organism, with particular reference to carbonic anhydrase, Physiol. Rev. 29: 370, 1949.

any group of metallic character. The study of the connection between zinc, insulin, and diabetes is still highly relevant, however, due partly to this metal's strengthening and prolonging effect on insulin, and partly to the recently published investigations by WOLFF (1951) describing how diabetes can be induced in animals by injecting a substance which forms an insoluble complex with zinc.

The metal is also of interest in *cancer* research since CRISTOL (1923) showed its concentrations in cancerous tissue to be strikingly high. The serum zinc of patients with malignant tumours appeared, in an investigation by VIKBLADH (1951), to be slightly lowered.

During the last ten years, zinc has come to the fore in medicine and biochemistry and a number of studies on the subject have been published. The rapid development of micro-analytical technique now makes possible the determination of minute quantities of the metal. The evolution of the isotope technique has also provided new methods in the study of the metabolism of zinc in the organism.

Thus it has been found that zinc is an essential component of the human organism, and that variations occur in physiological and pathological conditions.

Distribution of zinc in the organism

An adult human being usually consumes between 10 and 15 mg zinc a day, in certain cases considerably more (MCCANCE & WIDDOWSON 1942). The relative abundance of zinc in all normal foodstuffs renders "zinc deficiency disease" in man or animal very rare. In only one investigation, made by EGGLETON 1940) on Chinese subjects suffering from beri-beri, lowered zinc values in tissues and blood were found. With considerable difficulty laboratory animals have been kept on a diet so poor in zinc as to induce deficiency symptoms, manifested primarily by impaired growth in fur and epithelial formation and markedly retarded growth. If the food was supplemented in time by small doses of zinc, the deficiency was met (STIRN *et al.* 1935).

The total amount of zinc in the body is calculated at about 2 g (*cf.* FEARON 1946), *i.e.* about as much as the iron, and much more than the copper total. Although only about 2 % (30—40 mg) of the total body zinc is found in the blood, it is the blood zinc and its variations that have attracted most interest. The latest investigations on the subject (BERFENSTAM 1949, VIKBLADH 1950, WOLFF 1950) show that the plasma zinc amounts to about 0.1 mg %, and that of the blood corpuscles about 1—1.5 mg %. The strongest concentration appears to be in the leucocytes (DÉLEZENNE 1919, VALLEE *et al.* 1948), which according to VALLEE contain 3 % of the total blood zinc.

Even before radio-active zinc isotopes came into use, experiments had been made to study zinc metabolism in the body. The most enlightening results are those of MCCANCE & WIDDOWSON (1942). They found from balance tests on a series of subjects that the bulk of the zinc consumed in food was eliminated through the bowels, while the urine zinc was low. More zinc in the food gave more zinc in the faeces, while that in the urine remained low, only reaching high figures in cases of albuminuria.

MCCANCE and WIDDOWSON (1942) have also studied the effect of intravenous zinc dosage on human subjects. Here, too, the urine zinc was surprisingly little affected. The total elimination of the metal was increased, though the entire dose could never be recovered. This would indicate some regulating mechanism, though the degree of additional zinc storage in the tissues depends on the amount already present.

The metabolism studies made with the help of radio-active zinc, Zn^{65}, SHELINE *et al.* 1943, VALLEE & FLUHARTY 1950) can be briefly summarised thus: Nearly all radio-active zinc given intravenously will disappear rapidly from the plasma (*i.e.* within 10 hours), though certain of the tests indicate that the effect of such zinc may be traced for months. The zinc of the erythrocytes increases, at any rate during the first week following the injection. The liver clearly acts as a temporary storage place, zinc from the blood being deposited at such a rate that 1/3 of the dose is found in this organ 3 hours after injection. Practically all this has disappeared after a week, however, the metal having

apparently been absorbed into other organs, since only small quantities of the isotope are excreted.

Distribution of the blood zinc

Several publications have appeared dealing with the variations in the zinc content of the serum. VIKBLADH (1951) and WOLFF (1951), for example, have found abnormalities in certain pathological conditions, notably the low serum zinc values in infectious states accompanied by fever. VIKBLADH has also shown that the zinc in the serum protein is bound to at least two different components, more firmly to the one than to the other.

As regards the variations in the zinc of the erythrocytes only a few investigations are available. VALLEE and co-workers (1949) report increased values of corpuscle zinc in untreated cases of pernicious anaemia, and a tendency of the metal to decrease during subsequent treatment. The zinc in the erythrocytes is, nevertheless, of special interest, since it is now known that the erythrocyte zinc is, at all events in part, combined in a protein with enzyme function, namely *carbonic anhydrase*, which acts as a catalyst in the building up and breaking down of carbonic acid. This enzyme was discovered in 1932 (BRINKMAN, MAGARIA, MELDRUM, and ROUGHTON), and has since been intensively studied both for its biological significance and occurrence, and in order to reveal its chemical structure. KEILIN & MANN (1940) have shown that it contains about 0.3 % zinc, and similar results have been obtained by other investigators.

The enzyme has been found in most of the tissues and organs of the body (*cf.* VAN GOOR 1948). In the blood, its activity is demonstrable only in the erythrocytes, abnormal values being found in certain pathological conditions (LAMBIE 1938, HODGSON 1936) and during infancy (STEVENSON 1943, DAY & DU PAN 1947, BERFENSTAM 1948, JONES & WIDDOWSON 1949). It has been suggested that all the zinc in the body may be located in the enzyme, but investigations have proved that this is not the case (LEINER & LEINER 1941). The metal is undoubtedly present also in other forms. As mentioned above, the blood shows a zinc content of about 0.1 mg % in the plasma and a much higher con-

tent in the leucocytes, but in neither of these elements can any enzyme activity be demonstrated. The erythrocytes, on the other hand, reveal a certain parallelism between enzyme activity and zinc content (VALLEE *et al.* 1949, BERFENSTAM 1949).

It has been questioned whether the zinc content in the blood corpuscles — the carbonic anhydrase activity — can be influenced by excess zinc in the food. In the above mentioned experiments in which zinc deficiency in animals was induced by reducing their zinc supplies, the strongly affected animals were found to show no change in the activity of the carbonic anhydrase (HOVE *et al.* 1940). However no zinc analyses seem to have been performed in this connection. There are no reports of attempts to study the zinc content of the corpuscles — or the carbonic anhydrase activity — after dosage with extra zinc in the food apart from the present author's preliminary results of zinc tolerance tests on rabbits 1950. In the present investigation the author has tried to illustrate this problem by further experiments.

The present investigation deals with the following items:

1. Determinations of the variations in the zinc content of plasma and blood corpuscles in different age groups. This is of particular interest as the author earlier showed that the activity of the zinc-containing enzyme carbonic anhydrase varies with the age of the subject. It is low in newborn, especially in premature infants, and increases during the first year of life.
2. Determinations of the zinc content in infants' food to discover if this is inadequate and if the low zinc values found in infants can be caused by low zinc intake.
3. Determinations of the zinc content in the blood of pregnant women, foetuses, and newborn, to get some idea of the passage of zinc from mother to foetus.
4. Studies into the means of increasing the zinc content of plasma and blood corpuscles *in vitro* and *in vivo*, and, in case it is possible to increase the zinc content of the corpuscles, to study the changes in the activity of the carbonic anhydrase.

CHAPTER II

Methods for Determining Blood Zinc

A survey of the literature concerning determinations of zinc in blood shows that the different investigators have obtained very different normal values. Some of these are shown in Table I.

Table I. *Normal plasma- and corpuscle-zinc values in adults*

Investigator	Year	No. of cases	Zinc in plasma µg %	Zinc in corpuscles µg %
Burstein	1929		333	758
Smirnov	1948			1 300
Vallee & Gibson	1948	15 M.	390 (120—1 140)	1 420 (910—1 840)
		16 F.	310 (120—1 010)	1 450 (1 130—1 840)
Berfenstam	1949	19—24	113 (72—160)	1 303 (1 130—1 600)
Wolff	1950	50 M.	197 (137—284)	
		50 F.	194 (141—272)	
		10 M.		1 300 (1 101—1 501)
		10 F.		1 295 (1 101—1 497)
Vikbladh	1950	18 F.		1 783 (1 423—2 093)
	1951	50 M.	125 (84—159)	
		50 F.	123 (88—163)	

The difference in the results is probably due to differences in technique both in the *preparation* of the samples and in the actual *analysis*. Earlier investigators usually prepared their samples by dry ashing in quartz crucibles, or by wet ashing in Pyrex flasks. The analysis has been performed in different ways, *e.g.* polarographically (KOGA 1934, SMIRNOV 1948) colorimetrically with urobilin (LUTZ 1925), or by the potassium ferrocyanide method (FAIRHALL & RICHARDSON 1930). During recent years a colorimetric method with diphenylthiocarbazone (FISHER 1936) has been almost exclusively used.

Author's method

Principle [1, 2, 3]

The zinc was extracted from the protein in both plasma and corpuscles with strong hydrochloric acid, the proteins being then removed by precipitation with trichloroacetic acid.

Zinc determinations have been made with the 'dithizone method' introduced by FISHER in 1925. If a solution of diphenylthiocarbazone (dithizone) in carbon tetrachloride is shaken with solutions containing metals combining with dithizone, the metals pass over into the CCl_4-phase as coloured dithizone compounds. Separation of the metals is accomplished by varying the acidity of the solution, or by adding complex forming agents. The equilibrium between metal ion and metal dithizonate is reversible. Zinc, for example, which is most easily extracted at a pH of about 8, returns quantitatively to the water-phase at pH 2. This circumstance has been used to separate zinc from copper, the latter metal only going over to the water phase in strongly acid solution.

Zinc is separated from certain other metals by performing the extraction in the presence of thiosulphate as a complex-former.

Solutions of zinc dithizonate have a red colour, which allows the concentration to be determined colorimetrically.

Reagents and solutions

For preparation of the sample:

1. Hydrochloric acid 20 %
2. Trichloroacetic acid 20 %
3. Ammonium hydroxide (see below).

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[1] The author is greatly indebted to laborator FOLKE NYDAHL for valuable advice and help regarding the analytical procedure.

[2] For further details, see SANDELL: Colorimetric determination of traces of metals. 2nd Ed. New York 1950.

[3] The statistics of the series have been worked out by the usual methods. It can however be pointed out that the difference between two figures has not been considered statistically significant unless it was at least 3 times its standard error.

References: DAHLBERG, GUNNAR, Statistical Methods for Medical and Bio-

For analysis:

1. Dithizone dissolved in carbon tetrachloride.
2. Citrate buffer pH 8.5—9.0
3. Hydrochloric acid 0.02 N
4. Acetate buffer pH 4.75
5. Sodium thiosulphate 2 %.[1]

Water, passed through a cation exchange column, Amberlite IR 100, was used to prepare the solutions and for cleaning.

A commercial *hydrochloric acid* (LKB brand for serum iron determination) proved pure enough to be used in the analysis, but the *trichloroacetic acid* had to be vacuum-distilled in Pyrex glassware. Zinc-free *ammonium hydroxide* was made by dissolving ammonia in zinc-free water: the easiest method being to place a dish of zinc-free water in an desiccator containing ammonium hydroxide, d = 0.91, under slightly reduced pressure for some hours.

Dithizone solution: Stock solution: 50 mg dithizone in 500 ml carbon tetrachloride. Dilute solution: 40 ml stock solution diluted to 500 ml with carbon tetrachloride.

The carbon tetrachloride was "chemically pure" and free from sulphur, but had to be distilled before use. The solution was kept in a refrigerator. The concentration was checked daily by determining the *extinction* at 6000 Å.

Citrate buffer: 112 g secondary ammonium citrate is dissolved in water to make 1000 ml. The solution is adjusted to a pH of 8.5—9.0 with ammonium hydroxide and rendered zinc-free by shaking in a separating funnel with dithizone solution, and then several times with pure carbon tetrachloride.

Acetate buffer: Equal parts of 2-N sodium acetate and 2-N acetic acid are mixed, and purified as for the citrate buffer.

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logical Students. London 1940. — Fisher, R. A., Statistical Methods for Research Workers. London 1948. — Fisher, R. A. & Yates, F., Statistical Tables. London 1948.

[1] A 20 % sodium thiosulphate solution was first used. Thiosulphate of sufficiently low zinc content became impossible to procure, however, but as a ten times smaller amount of thiosulphate was found perfectly adequate, a concentration of only 2 % was adopted.

Glassware: Except for the pipettes of soda glass only Pyrex glass was used. The samples were taken and prepared in specially made tubes with ground stoppers.

Great care was taken in the *cleaning* of the glassware. Test tubes, centrifuge tubes and pipettes were thoroughly washed, and allowed to stand overnight in dilute nitric acid, after which they were rinsed with zinc-free water. With new vessels this procedure had to be repeated several times.

Sample-taking

The samples were taken in tubes with dry heparin. ('Heparin Vitrum' proved free from zinc.)

Venous blood from adults and older children was obtained by puncture with a stainless steel winged needle.

The first portion of the blood was allowed to run off into another tube, before the sample for zinc determination was taken.

On infants, the analyses were usually made on capillary blood taken from the heel after the skin had been carefully washed.

Preparation of the samples

Plasma.

When the sample was large enough, 2 ml plasma was used. This was diluted with an equal quantity of zinc-free water before the acid — 2 ml 20 % HCl — was added.

The sample was kept at 37° C for an hour, precipitated with 2 ml trichloroacetic acid, and centrifuged after standing for 10 minutes. The analysis was continued on the clear supernatant fluid.

Whole blood.

1 ml whole blood was haemolysed with three times the amount of water, and the sample then treated as the diluted plasma.

Corpuscles.

When analysing the zinc content of the blood corpuscles, difficulty of measuring the blood-cell mass was overcome as follows. A haematocrit reading was made, and a measured quantity — usually 1 ml — of whole blood pipetted off. The corpuscles were centrifuged and washed twice with zinc-free physiological

saline, after which zinc-free water was added up to the original volume (weighing before centrifuging and after washing).

The corpuscles thus prepared, were then haemolysed with three times the volume of water, and treated as above. After analysis the zinc content per ml "packed cells" was determined with the help of the haematocrit value.

The haematocrit determinations were made as double determinations in micro pipettes supplied by *Wifug Ltd.* for use with their centrifuge "T original", 4 800 r/m for 30 minutes.

Analysis.

The water-clear centrifuged sample, neutralised with NH_4OH, was poured into a separating funnel containing 20 ml dithizone solution and 10 ml citrate buffer, and shaken for about 2 minutes. Copper, zinc, and other possibly incidental metals combining with dithizone are thus bound as dithizonates, while iron is bound in a complex form. The carbon tetrachloride layer was transferred to another separating funnel containing 10 ml 0.02 N-HCl and shaken for 2 minutes. At this pH zinc dithizonate is dissociated, and zinc is quantitatively recovered in the hydrochloric acid solution, while the copper remains as dithizonate. The clear HCl solution was drawn off into another separatory funnel and shaken with 20 ml dithizone solution, and 5 ml acetate buffer, together with 1 ml sodium thiosulphate solution. This means that zinc is once again combined with dithizone, while the remaining metals (cobalt, nickel *etc.*) are complexly bound to thiosulphate.

The extinction was determined using the dithizone solution as reference solution in a Hilger "Spekker Photoelectric Absorptiometer" with green filter (maximal transmission at 5 200 Å.).

The calibration curve was constructed using standard solutions made from reagent-grade zinc or zinc sulphate. In the region used here, the curve followed a straight line. This region extended up to 5 μg zinc.[1]

[1] To start with, a modified method with less dithizone solution was used when the amount of zinc was expected to be very small, but the method described here proved exact enough even for quantities as small as 0.5 μg.

To ensure that the solutions, reagents, and water used were sufficiently pure, control tests were run for every series. Only blanks corresponding to a maximum zinc content of 0.2 μg were accepted.

The Accuracy of the Method

The accuracy of the method of determination used in the investigation has been checked by analysis of known, varying amounts of zinc as zinc sulphate solution. The results of these determinations have been entered in Table II.

Table II. *Determinations of zinc in zinc salt solution.*

Amount of zinc in μg					
Calculated	Obtained	Calculated	Obtained [1]	Calculated	Obtained [1]
2.00	2.00	1.50	1.48	1.00	0.98
2.00	2.00	1.50	1.49	1.00	1.01
2.00	2.00	1.50	1.49	1.00	1.00
2.00	1.99	1.50	1.50	1.00	1.01
2.00	2.01	1.50	1.48	1.00	1.01
2.00	2.02				
2.00	2.00				
2.00	2.00				
2.00	1.99				
2.00	2.02				

To test the accuracy of the method of preparation a series of experiments was carried out. In Table III results are given from analyses on plasma and whole blood obtained both by the author's extraction method and by ashing. The ashing process was carried out in platinum crucibles at 500° C for 24 hours, after which the crucibles were boiled out with hydrochloric acid. The values obtained correspond very well with each other although slightly higher values are usually obtained by extraction with acid.

In order also to obtain information on the reproducibility of the method of extraction the author has made statistical calcula-

[1] Determinations performed with reduced amounts of reagents.

Table III. *Comparison between values for the zinc content of plasma and blood corpuscles obtained by ashing in platinum crucibles, and by extraction with acid.*

Zinc in plasma µg %		Zinc in whole blood µg %	
Extracted with acid	Ashed	Extracted with acid	Ashed
127	110	650	600
145	115	750	640
112	125	620	680
78	70	720	710
113	93	685	670
115	140	725	675
100	116	691	610
		689	590
		586	565
		510	500
		560	540
		627	520
Mean 113	110	651	608

tions on a series of 28 double determinations on plasma. The error of the method[1], σ_m is 3 µg %, *i.e.* about 3 % of the mean of zinc in plasma. This means that the plasma zinc value obtained by this method practically never deviates more than 10 µg % and, with the probability 0.95 deviates less than 6 µg %, from the "real" value. In the same way a calculation has been made on 14 double determinations on whole blood and blood corpuscles with a zinc content of 200—600 µg %, and 12 double determinations on blood corpuscles with a zinc content of 1 200—1 800 µg %, in order to work out the corresponding error of the method. In the former σ_m was 10 µg %, and in the latter 30 µg %. If, however, the difference between the two determinations is expressed as a percentage of the mean, σ_m is about 2.5 % in both cases. This means

[1] According to the formula $\sigma_m = \sqrt{\frac{\Sigma d^2}{2n}}$, where d represents the difference between the two determinations and n the number of differences.

that the corpuscle zinc value obtained by this method practically never deviates more than 7—8 % from the 'real' value. The method can be considered satisfactory.

Discussion

Since our analyses included whole blood and corpuscles, all the blood samples were taken with heparin. It is thus *plasma* and not *serum* that has been analysed.

In a small number of cases the author has determined the zinc content both in plasma and serum from the same subject. The results are given in Table IV.

The series is short, but seems to show clearly enough that the plasma and serum zinc levels are the same. The difference present in a certain case is not larger than can be explained by the error of the method. Similar conditions prevail regarding serum and plasma iron (VAHLQUIST 1941).

During the first part of the investigation, if the samples were large enough, control tests were made on plasma and corpuscles as well as on whole blood. In an earlier paper (1949) the author has reported a series of values for whole blood, partly obtained

Table IV. *Comparison between the zinc content of plasma and serum from the same subject.*

Case no.	Zinc content	
	Plasma µg %	Serum µg %
1	116	112
2	102	107
3	118	117
4	113	115
5	136	128
6	113	116
7	104	104
Mean	114.5	114.1
Difference	—0.4 ± 1.7	
Standard deviation (σ)	4.4	

by direct analysis, and partly calculated from the corpuscle and plasma zinc with the aid of haematocrit readings. Measured and calculated values were found to agree closely. In connection with the present investigation a series of 49 similar determinations and calculations has been carried out. In the 49 cases the mean of the measured values of the zinc content was 575 µg %, and the mean of the calculated values 573 µg %. The difference is 2 ± 5 µg %. The standard deviation of the series of differences is 36 µg %, *i.e.* about 6 % of the mean. The agreement between the values obtained from the same blood is considered satisfactory.[1]

The author is aware that, with his methods, the corpuscles will include not only erythrocytes but also leucocytes. Although VALLEE & GIBSON (1948) have shown the leucocytes to have a high zinc content in comparison with the erythrocytes (25 times as much in man and even more in rabbits), they are so few (1 to 500—1 000 erythrocytes) that they do not appreciably affect the values concerned.

The present author has shared the difficulties of other investigators in the matter of procedure. During the first few months of this investigation, the previously described methods of ashing were used. The results obtained, both by dry ashing in quartz crucibles and by wet ashing with perchloric acid and sulphuric acid in Pyrex flasks, were found to be inaccurate, and both methods had to be abandoned. Known amounts of zinc could not be recovered quantitatively by this route, possible explanations being that, on ashing, the metal may become bound to the material in the crucible, or it may distil off on account of its low boiling point.

To avoid these difficulties, investigations were made to see whether the zinc present in plasma and formed elements of the

[1] This is undoubtedly not influenced by the fact that the corpuscle zinc values in umbilical cord blood are likely to be somewhat less reliable than those in the rest of the investigation. Unfortunately, the zinc values have, in nearly all cases, been deduced from the plasma- and whole-blood-values. A simple calculation thus shows that in these cases, with their relatively high plasma zinc, very low whole-blood zinc, and high haematocrit values, the corpuscle zinc values are somewhat less reliable than the corresponding values obtained in older children and adults.

blood could not be extracted without destroying the organic substances.

It is known, that copper and iron can be extracted from the plasma with the aid of acid, and this suggested similar possibilities in the case of zinc. Concerning iron and copper in the corpuscles it is known that all the copper but only a small amount of the iron is removable by the same method.

When the author had worked out a method involving extraction of zinc with acid VALLEE & GIBSON (1948) published an investigation on blood zinc in which they state that platinum is the only material suitable for ashing crucibles. It was therefore possible to test the extraction method by running it parallel with ashing in platinum crucibles. *It was found that the zinc in both plasma and corpuscles was completely extracted by the acid.*

The advantages of this extraction method are obvious. Smaller samples may be used, the procedure is much shorter and needs no extra equipment, such as platinum crucibles, oven, *etc.* A great advantage is the elimination of the risks of contamination during ashing. Moreover, it avoids the difficulties of a quantitative removal of the ashed material from the ashing vessel. The latter procedure involves a number of boilings with acid, *etc.*

HOCH & VALLEE (1949) have also evolved a method for extracting zinc with acid, in which the samples are treated three times with trichloroacetic acid. They claim to have found slightly higher zinc values for each extraction. This method does not seem to have any advantage over the simpler one of the present author.

VIKBLADH (1950) has also tried out the extraction of zinc with acid, but reports slightly lower values than those obtained after ashing. If, however, the acidified samples are heated to 90° C for 1—2 hours the values are then, according to the same investigator, exactly the same as those obtained from ashing. The discrepancy probably does not arise from the heat treatment, however, since the present author has made a series of determinations on samples before and after warming, with identical results.

Both for blood corpuscles and plasma, it has proved advisable to reduce the protein concentration by adding water in order to prevent the formation of a compact precipitate. By shaking in the

glass-stoppered Pyrex centrifuge tube the precipitated proteins are finely dispersed after acidification. The subsequent precipitation with trichloroacetic acid and centrifuging can then be done in the same tube.

As mentioned above, the colorimetric method adopted carefully guards against any disturbance of the final colour reaction from other metals in the biological material analysed. To ensure that variations in their concentrations have no effect, zinc determinations were made in the presence of considerable quantities of iron and copper. The result of the analysis was unchanged.

There are, however, certain differences between the present author's method and that described by VALLEE & GIBSON (1948) and also used by VIKBLADH (1950). The differences apply to some extent to the buffer solutions, but the main difference lies in the colorimetric procedure. VALLEE & GIBSON prescribe an arbitrary amount of dithizone solution, and the value is then calculated from colorimetric readings both of the green dithizone residue and of the red dithizonate formed. The present author, on the other hand, pipettes off an exact volume of dithizone solution (containing a large dithizone excess for a quantity of zinc up to 5 μg), which demands only one reading. The validity of this single reading has been questioned (VIKBLADH) on the grounds that the concentration of the dithizone solution may be altered by evaporation or bleaching. In the present case evaporation has been kept as low as possible by using stoppered vessels. A daily colorimetric check of the colour was always performed. The author also tried the method of VALLEE & GIBSON, and found values which agreed with those obtained by his own method but were subject to a considerably greater dispersion.

CHAPTER III

Binding of Zinc in the Blood

The total zinc in the adult human body has been estimated at 2—3 g (*cf.* FEARON, 1946). The blood zinc constitutes only a very small portion of this, *i.e.* about 30 mg, of which 9/10 is found in the corpuscles. The plasma, therefore, only contains 3—4 mg, or about 1/1000 of the body total — about the same proportion as that shown by serum iron to total iron. The incidence and distribution of the three metals, iron, copper, and zinc, are tabulated in Table V.

Table V. *Some figures for iron, copper, and zinc, in the human body.*

	Daily Intake	Total Amount of Metal in Human Body	Serum Value	Corpuscle Value	Intracorpuscular Protein-bound Metal in
Fe	10—25 mg	3—5 g	120 µg %	100 000 µg % (including 2 000—4 000 µg % "easily split off iron")	Haemoglobin 0.34 % Fe
Cu	2—5 mg	0.1—0.15 g	120 µg %	About 100 µg %	Haemocuprein 0.34 % Cu
Zn	10—15 mg	2.2 g	110 µg %	1 300 µg %	Carbonic anhydrase 0.33 % Zn

A. Binding of Zinc in the Plasma

While the physico-chemical properties of serum copper and serum iron have been studied in detail, so far only one investigator

has really gone into the question of serum zinc, namely VIKBLADH (1951). She considers that zinc is normally present in the serum in at least two separate fractions, in a ratio of about 1:2 — one, probably of globulin character, where the metal is firmly bound, and one with loosely bound zinc, precipitated as albumin. The latter larger, fraction would, in her view, represent the transport zinc.

The present author's investigations into the stability of the zinc-protein complex *in vitro* were performed in connection with the working out of the analytical procedure. Studies on the zinc-binding capacity of the plasma provide a theoretical background for the experiments on animals. The binding of zinc to the serum proteins has a practical interest in the investigations into the plasma zinc of children and adults and the transport of zinc through the placenta.

The pH stability of the plasma zinc-protein complex

As in the serum iron analyses, it was found possible to extract also the serum zinc with acid. This was checked by parallel ashing and extraction tests (Chapter II). As a supplement to this investigation it was felt desirable to study the pH stability of the zinc-protein complex.

To determine the amount of zinc ionized at a given pH, small portions of the same plasma were adjusted with hydrochloric acid to different pH values, and dialysed against physiological isoacid sodium chloride solutions by the method previously described by VAHLQUIST (1941). The acid diluted with physiological NaCl was added to the inner fluid. The ratio between the volume of this mixture and the plasma volume was 1:4. The dialysis tubes were washed in weak hydrochloric acid before use. Dialysis was performed at about +4° C for 24 hours. The outer fluid, whose volume was about 25 times that of the inner, was stirred occasionally. The tubing with contents was weighed before and after dialysis. The pH values were measured with a glass electrode on a portion of the inner fluid *after* the dialysis was completed. Another portion was taken for zinc analysis, which

was performed as described in Chapter II. In some of the experiments physiological NaCl was dialysed in one of the tubes to determine the blank value.

Results

The results are shown by the diagram in Fig. 1, where the percentage of non-dialysed zinc is plotted against the pH values. The zinc-protein complexes are seen to be stable in physiological pH range but begin to break up when the pH is lowered, the process being apparently complete at about pH 4. The amount of zinc (about 20 μg%) remaining at lower pH values does not significantly exceed the blank value. The slight increase in weight of the inner fluid has been disregarded in the calculations, even though in a few cases it reached a figure of nearly 10% — this, naturally enough, at the lowest pH values, when the zinc content in the inner fluid was so low that a correction by 10% would have no practical significance. The precision of the method is also lower at this low zinc content, so that a difference of this magnitude is quite within the limits of error.

Tests concerning dialysis of one sample of plasma at alkaline pH values revealed that the zinc did not begin to separate until the pH was over 10, and that half of it still remained bound even at pH values larger than 11.5.

Discussion

VAHLQUIST's investigations (1941) have shown that the iron-protein complex is stable within a broad zone down to a pH of 4.5, and that only at a pH-value of between 1 and 2 does so little iron remain as to suggest that the ionization process is complete. LAURELL (1947), using a different technique, on the other hand considers that iron can be demonstrated in ionized form already when the pH has fallen slightly below the physiological.

Opinions have also differed on the stability of the copper-protein complex. BOYDEN & POTTER's earlier investigations (1937—38), showing that though ionization began at pH 6 it

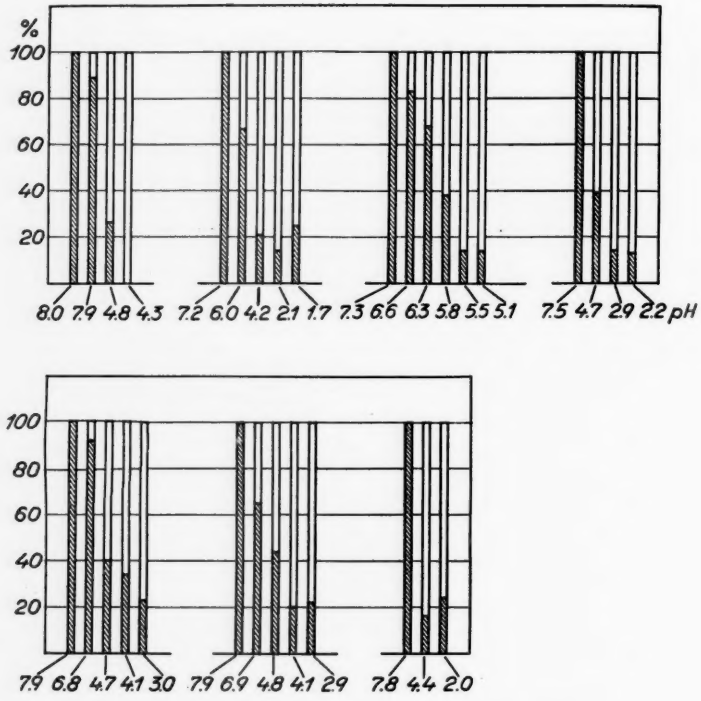

Fig. 1. *Results of dialysis trials at different pH-values.*

Each group of columns represents dialysis of the same *plasma* at different pH-values. The shaded part represents the non-dialysed fraction given as a percentage of the total zinc content.

had not reached further than 50 % at pH 1.6, are somewhat contradicted by KEIDERLING's report (1950) that ionized copper is found at pH values just below the neutral point.

Of the three metals mentioned here zinc seems under all circumstances to be the one most easily separated from its protein carrier by the action of hydrogen ions.

Capacity of the plasma protein to bind further zinc

In the physiological pH range all the zinc in the plasma is combined with the proteins and cannot be removed by dialysis. It was not known, however, whether the plasma protein could bind an extra addition of the metal, and it was felt such information might be useful when planning zinc dosage tests on animals.

Dialysis experiments were accordingly performed on plasma enriched *in vitro* with zinc. Some experiments were also performed on pure serum albumin.[1]

Methods

The plasma portions were supplemented in vitro with different amounts of zinc in the form of zinc sulphate, usually in a volume of not more than 5 % of that of the plasma. The plasma sample was shaken during the addition, to prevent local precipitation. The samples were transferred after about 2 hours to the dialyser tubes from which a pre-dialysis sample was taken, to give the initial value of the zinc content. The samples were dialysed for 24 hours at 4° C against plasma or physiological NaCl. In the latter case, the liquid was changed several times during the course of the experiment. A 4 % solution of albumin was used—*i.e.* a solution with about the same albumin concentration as in plasma — and subjected to the same procedure.

Results

Table VI shows the zinc content in zinc-enriched plasma before and after dialysis. In some of the tests there is no difference between these two values, while others show that small amounts of the metal have been dialysable.

Discussion

Plasma is obviously able to bind large quantities of extra zinc, and no upper limit for this capacity has been found in these experiments. It has been possible to reach plasma zinc concentrations which are more than one hundred times higher than normal.

[1] Concentrated human serum albumin prepared according to COHN, supplied through the courtesy of laborator KAI O. PEDERSEN.

Table VI. *Power of plasma to combine with amounts of zinc above the normal at physiological pH.*

Dialysis of zinc-enriched plasma with physiological NaCl solution and plasma.

Plasma I		Plasma II		Plasma III		Plasma IV		Plasma V		
µg % zinc before dialysis with NaCl	µg % zinc after dialysis with NaCl	µg % zinc before dialysis with NaCl	µg % zinc after dialysis with NaCl	µg % zinc before dialysis with NaCl	µg % zinc after dialysis with NaCl	µg % zinc before dialysis with NaCl	µg % zinc after dialysis with NaCl	µg % zinc before dialysis with NaCl	µg % zinc after dialysis with NaCl	µg % zinc after dialysis with plasma
105	128	390	570	520	518	550	424	860	780	820
206	215	680	540	670	650	735	615	1 540	1 410	1 540
325	290	730	580	840	840	1 040	790	2 240	1 960	2 040
535	360	920	720					8 400	7 100	7 350
610	520	910	720							
896	760									

In some of the experiments, however, it seems as though a small quantity of the metal responded to dialysis. This may possibly be attributable to technical details in connection with the zinc additions. In all probability it is *not* due to the exceeding of a saturation limit — as in other tests zinc was assimilated to many times the original amount.

Another proof of this capacity of the plasma protein is found in zinc tolerance tests on rabbits (Chapter VIII). If any great part of this injected zinc were in ionic form, it would rapidly be eliminated from the blood stream. The tests showed, on the contrary, that the zinc in plasma, enriched *in vivo* was totally undialysable, although the samples were taken immediately after intravenous injection of the zinc salt. The slow fall in the plasma zinc curve after intravenous loading, leading to strongly raised values for some hours following dosage, is another indication that large quantities of the metal can be bound to the protein.

The zinc-enriched plasma was dialysed either against physiological NaCl or plasma. No difference in the results was obtained in either case.

Table VII. *Power of albumin solution to combine with amounts of zinc above the normal at physiological pH.*

Dialysis of zinc-enriched 4 % albumin solution with physiological NaCl solution.

µg % zinc before dialysis	µg % zinc after dialysis
320	364
685	626
1 150	1 030

Whereas copper cannot be assimilated to serum in amounts larger than the physiological quantity (HOLMBERG & LAURELL 1947, KEIDERLING 1950), and the serum has a saturation limit (LAURELL 1947) for iron, plasma seems to have an inordinate avidity for zinc. The mode of combination of zinc with the plasma proteins is therefore of interest.

It appeared from dialysis of zinc-enriched albumin solution that the albumin in a solution corresponding in strength to that in the plasma was able to bind large quantities of zinc. This experiment, however, does not prove definitely whether it is the albumin alone that binds the additional zinc, or whether the globulins also take part in the process. Table VII.

It would, of course, be extremely interesting to separate the serum proteins and study the zinc content in the different fractions. The present author has carried out a few single tests with the familiar method of precipitating with half-saturated ammonium sulphate solution, 60—70 % of the zinc being then recoverable in the filtrate, *i.e.* in the albumin fraction (BERFENSTAM 1952). This held both for untreated plasma and for samples enriched *in vitro* and *in vivo*. Precipitation tests of this kind are no sure evidence of the binding of the zinc, however, since the ammonium sulphate gives no distinct separation into albumin and globulin. The results also agree with those submitted by VIKBLADH in 1951.

An ordinary electrophoretic separation of non-supplemented plasma would naturally be the most practical route for discovering where the zinc is bound under physiological conditions, but technical difficulties made this investigation impossible. Instead, the author tried paper-electrophoresis with the increased possibilities of separation which this method offers, using serum enriched with zinc to give an analysable quantity in each fraction.

The tests indicated that 60—70 % of the total zinc in the enriched

serum is bound in the albumin. However, the fact that the zinc-protein ratio is the same for both albumin and total globulin — the method does not allow of any separation of the different globulins — rather indicates that, proportionately, the metal is evenly distributed over these protein fractions.

There is always the possibility that the above only holds for a serum that has been overloaded with metal, and it might possibly not apply to serum with the normal low zinc content.

B. Binding of Zinc in the Corpuscles

There were certain reasons that the investigations of the pH stability of the zinc-binding protein component in plasma and this proteins' capacity to bind further quantities of zinc should be repeated for corpuscles. One of the motives for the first of these was that here, too, a method based on acid extraction was used. In the second case, interest had been aroused by the peroral and intravenous zinc tolerance experiments on animals, which yielded a temporary rise of the zinc content not only in the plasma but also in the corpuscles. (Chapter VIII.)

Method

The blood samples were centrifuged and the corpuscles washed with physiological NaCl solution. They were then haemolysed in about 5 times the volume of water. In the investigation of the pH stability, the pH values were set and adjusted with an acid solution in the same way as for plasma, but the dialysis in this case was made against isoacid dextran solution.

The tests involving zinc additions were also made in the same way as for plasma, except that dialysis was made against dextran solution, which was exchanged during the course of the experiment.

Results

The diagram (Fig. 2) shows that when the pH is lowered from the neutral point towards a value of 2.5, increased quantities of ionised zinc are given off. A small amount of the metal is still found in the inner fluid at about pH 2.5.

Table VIII indicates that large quantities of zinc can be bound to the corpuscle protein at a physiological pH.

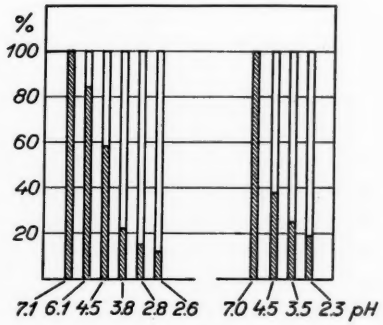

Fig. 2. *Result of dialysis trials at different pH-values.*

Each group of columns represents dialysis of the same solution of *blood corpuscles* at different pH-values. The shaded part represents the non-dialysed fraction given as a percentage of the total zinc content.

Table VIII. *Power of a solution of haemolysed blood cells to combine with amounts of zinc above the normal at physiological pH.*

Dialysis of zinc-enriched solution of haemolysed blood cells against 'Macrodex' (Pharmacia) solution.

µg % zinc before dialysis	µg % zinc after dialysis
550	650
1 000	1 000
1 600	1 600

Discussion

In the dialysis tests made to determine the pH stability of the plasma zinc protein, physiological NaCl was used as outer fluid. The volume of the inner fluid was thereby changed only slightly. In similar tests on haemolysed corpuscle solution, on the other hand, physiological NaCl proved unsuitable. Dextran solution lent itself very well to the purpose, however, since it proved to be practically free of zinc, to allow pH adjustment, and to produce a good osmotic balance.

Fig. 2 shows that the zinc complex loses its stability already before pH 6 has been reached, and that at about 4 the bulk of

the metal has been extracted. About 15—20 % is still demonstrable in the inner fluid at a pH of about 2.5. This probably represents the blank value.

Dialysis was not performed at lower pH values for technical reasons.

It appears, then, as if the stability of the zinc protein complex in the corpuscles differs very little from that of the plasma; and it is evident that the amount of acid used in the acid extraction procedure also in the case of corpuscle zinc is far greater than the amount necessary to break the zinc-protein complex.

We already know that copper can be separated from its protein complex in the corpuscles with the help of strong acid, while only a small proportion of the corpuscle iron — *i.e.* the so-called "easily split off iron" — responds to such treatment. As regards acid stability, then, the corpuscle protein complex of zinc seems to agree more with that of copper than with that of iron.

The dialysis tests made with haemolysed corpuscles and added zinc salt solutions gave results that suggest that the corpuscle protein is able to assimilate additional zinc. That this capacity is considerable is shown by the fact that the protein concentration in the solution used was estimated at only about 5 %, *i.e.* a seventh of that in the corpuscles, and that all the added zinc could be completely assimilated, despite a concentration of up to 1600 μg %. This is to compare with the results of the tolerance tests on rabbits (Chapter VIII), which yielded only a transient and moderate corpuscle zinc rise despite a very strong one in the plasma zinc.

This indicates that the zinc in the *in vivo* tests is bound immediately to the plasma proteins.

It should be stated here, that these experiments only give an approximate assessment of the pH stability of the zinc-protein complex in plasma and in corpuscles. It is almost impossible to avoid contamination in experiments of this kind, which call for a great deal of handling, and the method is not precise enough for the small quantities of zinc involved in dialysis at low pH values, so that an exact determination of the pH value at which zinc is completely dialysable is out of the question.

CHAPTER IV

Investigations into the Zinc Content of Plasma and Corpuscles from Adults and Children at Various Ages. The Correlation between the Zinc Content and the Activity of Carbonic Anhydrase in Corpuscles

As previously stated, the normal values for plasma and corpuscle zinc, reported in the literature differ very greatly (Table I). Certain of these normal values — and particularly those obtained by methods involving ashing in quartz crucibles — are now considered untrustworthy.

A. Zinc Content in the Plasma and Corpuscles of Adults

Though the present investigation was primarily concerned with the blood zinc of children, analyses have also been carried out on blood from healthy adults, for purposes of comparison.

Material

The determinations covered the years 1948—52. The material consisted of healthy nurses, medical students, and blood donors at the University Hospital, Uppsala. The blood donors were usually conscripts. Ages varied between 18 and 59 years. The samples were taken at all times of the year and, when not otherwise stated, before 11 a.m.

Results

The results are collected in Table IX. The series consists of 84 men and 42 women. The mean plasma zinc contents in men and women are practically identical, *viz.* 110 ± 2 and $108 \pm 4 \mu g\,\%$. However, the mean zinc content of the erythrocytes was somewhat higher in men, *viz.* $1\,286 \pm 24 \mu g\,\%$ against $1\,202 \pm 30 \mu g\,\%$

Table IX. *The zinc content of plasma and blood corpuscles in healthy men and women.*

Sex	Plasma			Blood corpuscles		
	No. of indivs.	Mean ± standard error µg %	Standard deviation µg %	No. of indivs.	Mean ± standard error µg %	Standard deviation µg %
Men	84	110±2	18	42	1 286±24	160
Women	42	108±4	25	42	1 202±30	195
Both sexes .	126	109±2	20	84	1 244±20	180

Table X. *The probability of the blood zinc in a healthy person lying between certain limits.*

Probability	Zinc content in plasma µg %	Zinc content in corpuscles µg %
90 %	75—140	950—1 540
95 %	70—150	890—1 600
98 %	60—155	825—1 660
99 %	55—160	780—1 710
100 %	50—170	700—1 790

in women. The difference is 84±39 µg % and is not statistically significant. The plasma zinc content for both sexes together averages 109±2 µg % and the corpuscular zinc 1 244±20 µg %.

Table X gives the probability for a single determination of the zinc content lying between certain limits in a healthy person.

In the majority of cases the values were checked by double determinations. When the analyses were performed only on plasma and whole blood, the corpuscle value was calculated with the aid of the haematocrit value (*cf.* Chapter II).

Table XI furnishes the values of the zinc in whole blood, plasma, and corpuscles from the same subject on several occasions during 14 months. As can be seen, the variations are insignificant.

Table XI. *Repeated zinc determinations on blood from the same subject during the course of fourteen months.*

Date	Haematocrit value	Zinc in plasma µg %	Zinc in corpuscles µg %	Zinc in whole blood µg %
21.1. 1949	41	154	1 380	650
22.1.		116	1 390	636
31.1.	42	132	1 350	640
3.3.	41	131	1 370	680
17.3.	43	128	1 450	700
1.4.	43	134	1 320	628
2.6.	42	120	1 460	660
30.7.	43	114	1 280	670
1.9.	41	134	1 360	648
14.11.	45	127	1 420	—
31.3. 1950	44	132	1 360	640

B. Zinc Content in Plasma and Corpuscles of Newborn Premature Infants, Newborn Full-term Infants and Children of Different Ages

Material and methods

The material consisted of premature and full-term infants and children of different ages, all treated at the Children's Clinic, Uppsala. Children with any symptoms of current infection, or such as were suffering from diseases of the blood have not been included. It was not possible to confine the taking of samples from this young material to any one time of the day. The technique used on adults — *i.e.* venous puncture with a winged needle — could only be applied to children over the age of 3—4 years. In other cases the analysis was performed on capillary blood, taken from the heel of the infants and the finger of those slightly older children, after the skin had been meticulously washed with ether and zinc-free alcohol. Precautions were taken to avoid any possible contamination of the specimen by zinc-containing powders and ointments.

To prevent haemolysis, and to enable the collection of the blood quantitatively, the blood was sucked up into pipettes washed in acid and treated with heparin, and was then transferred to previously weighed Pyrex test-tubes. Two different samples, where possible of 1 ml each, were taken from each child. The quantity was determined by weighing the tubes.

The samples were centrifuged and the plasma pipetted off and weighed. The corpuscles were washed and haemolysed, and both plasma and corpuscles then treated as described previously (Chapter II).

For assessing the blood zinc at birth, umbilical cord blood was analysed.

Subsequently, a similar investigation was made on a material of foetuses (Chapter VII).

Results

Table XII gives the values obtained from determination son premature newborn infants and newborn full-term infants (umbilical cord blood), infants and older children.

To elucidate the increase in the corpuscle zinc with rising degree

Table XII. *The zinc content of plasma and blood corpuscles in premature infants, umbilical cord blood, infants, children of various ages, and adults.*

Age groups	Plasma			Blood corpuscles		
	No. of indivs.	Mean ± standard error µg %	Standard deviation µg %	No. of indivs.	Mean ± standard error µg %	Standard deviation µg %
Premature infants	16	187 ± 20	78	21	347 ± 13	59
Umbilical cord blood .	39	125 ± 5	33	32	376 ± 19	110
First week of life.....	12	164 ± 15	52	15	411 ± 25	97
2nd week — 3 months.	11	96 ± 14	48	12	591 ± 51	176
4—11 months	15	127 ± 8	29	16	777 ± 43	170
1—5 years.....	11	130 ± 10	32	12	1 055 ± 44	152
6—11 years.....	12	113 ± 5	17	12	1 153 ± 51	177
Adults (both sexes) ...	126	109 ± 2	20	84	1 244 ± 20	180

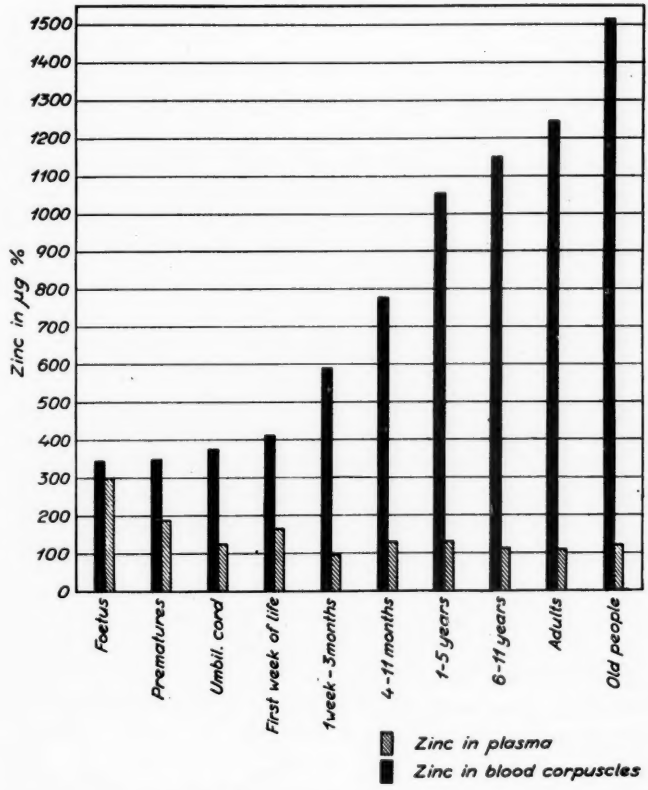

Fig. 3. *The zinc content of plasma and blood corpuscles in foetuses, newborn infants, children of various ages, in adults, and in old people.*

of maturity at birth, and subsequent development, the means for corpuscle and plasma zinc have been plotted on a diagram (fig. 3) which also includes values for foetuses, adults, and elderly people (over 60 years).

The author's series consists of 16 premature infants born 3—8 weeks before term. In several cases it was not possible to take blood samples for zinc analyses during the first few days of life owing to the infant's condition. In some cases the first sample

was taken in the 3rd week and in one in the fifth week. In all 16 cases the mean plasma zinc was 187 ± 20 μg %, and the mean corpuscle zinc 347 ± 13 μg %. If those infants where the first samples were taken after two weeks are excluded, 9 remain, and in these the mean plasma zinc was found to be 200 ± 29 μg % and the mean corpuscle zinc 354 ± 22 μg %. As this difference is small all 16 premature infants are included in the series.

The plasma zinc in umbilical cord blood of mature infants (125 ± 5 μg %) is considerably lower than in the premature infants. However slightly higher plasma zinc values than in the umbilical cord blood were found in normal babies during the first week of life (164 ± 15 μg %). Both this mean value of the plasma zinc and that in umbilical cord blood are significantly higher than the mean in adults. The different age groups show irregular variations in the plasma zinc levels with lowered mean values for the first 3 months of life (96 ± 14 μg %), rising during the latter part of the first year, and in the following years of childhood falling again towards the adult level. If the both groups with the highest plasma zinc are combined (*i.e.* 4 months—5 years) a mean value is obtained (128 ± 6 μg %) for this bigger series which lies significantly higher than the adult level (the difference is 19 ± 6 μg %).

The zinc content of the blood corpuscles is very low in the foetus (under 400 μg %) (Chapter VII), in premature infants, and also at term, after which it rises steadily with the age, and reaches about 800 μg % at the end of the first year. By 10 years of age it equals the adult value of about 1 200 μg %.

C. Correlation between Zinc Content and Carbonic Anhydrase Activity in the Erythrocytes

As mentioned in the introduction (p. 10), zinc is an essential component of the enzyme carbonic anhydrase. This enzyme activates the carbonic acid metabolism of the body and, in the blood, is present in the erythrocytes but not in the plasma.

Strikingly low values for carbonic anhydrase activity are found only in the newborn, particularly in the premature (Stevenson 1943, Day & Du Pan 1947, Berfenstam 1948, 1952). The

present author has also shown that this activity is proportional to the degree of maturity, and that it increases during the first year of life. It was also shown that an increase, probably due to an activation process, can be induced in the infants blood, after injection of blood — or even of plasma — from an adult.

The author has determined the enzyme activity in blood from children and adults using a modification of the Meldrum-Roughton boat-method. To obtain comparable values, the activity has been calculated per *corpuscle volume*, *i.e.* by computing the quotient between activity and haematocrit value.

The main concern of these studies was to discover whether there was any connection between enzyme activity and zinc content in the corpuscles of children. It was however impossible to do this until a suitable method for determination of the zinc content in the small quantities of blood obtainable from children was attained. In 1949 the present author was able to carry out a study of the correlation between zinc content and carbonic anhydrase activity (BERFENSTAM 1949). The values found are plotted in the diagram in fig. 4.

Discussion

We already know, that many of the results of earlier investigations suffered from technical weakness in procedure. It is also plain that some of them concerned a very small number of cases. Analytical errors are naturally most noticeable in analyses of plasma where the zinc content is low.

In this investigation the results of plasma zinc determinations in premature infants, the new-born and young children, must be considered to be less accurate than those in older children and adults, because capillary blood has been used. This is due partly to the increased risk of contamination in this method of taking samples, and partly to the fact that the analyses were sometimes made on very small quantities of plasma. There is little reason to doubt the results of the corresponding tests on the blood corpuscles, however. In all these tests the size of the specimen has been adequate, and in about 80 % of the cases double determinations have been performed.

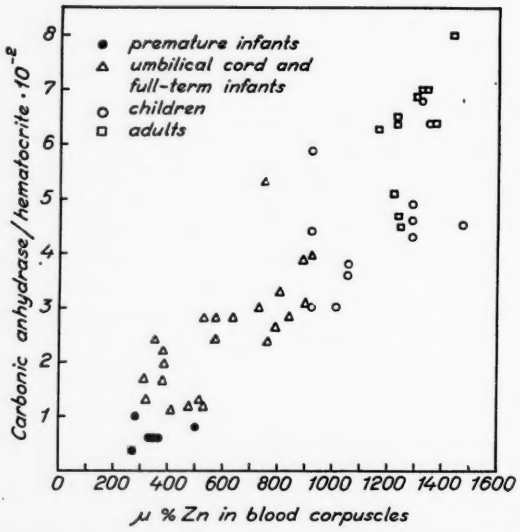

Fig. 4. *The relationship between zinc and carbonic anhydrase in blood from infants, children, and adults.*
The enzyme-values are given as the quotient of carbonic anhydrase in whole blood and the haematocrite value.

Several of the more recent publications show relatively good agreement in the corpuscle values: VALLEE & GIBSON's (1948) results have already been discussed (BERFENSTAM 1949). VIKBLADH's corpuscle values (1951) are somewhat higher than those of the present author's. WOLFF (1950) who prepared the samples by wet ashing reported values tallying with those now reported. As far as the present author knows, the earlier publications on this subject confine themselves exclusively to adults and in nearly all cases to normal individuals. WOLFF (1951), however, has studied the corpuscle zinc in certain pathological conditions, one result being that the values were found to be raised in latent or manifest respiratory insufficiency. VIKBLADH in 1950 has confirmed the earlier published results by the present author (BERFENSTAM 1949) concerning the low value of corpuscle zinc in newborn infants.

As regards plasma zinc VIKBLADH (1950, 1951) found good agreement with the results of the present author (1949), whereas WOLFF (1950, 1951) obtained values which were considerably (about 50 %) higher.

As appears from Table IX, the corpuscle and plasma zinc values of normal adults in this investigation showed only moderate variations. No statistically significant difference with the sex was obtained. As the work went on, it was found that the corpuscle values in old people were somewhat higher than the presumed normal figure.

In 13 elderly subjects (60—93 years) the mean plasma zinc content was 122 ± 6 µg % ($\sigma = 21$ µg %), and the mean corpuscle zinc $1\,515 \pm 47$ µg % ($\sigma = 170$ µg %).[1] The last mentioned value is here significantly higher than normal. The plasma zinc is also raised, but the difference is not statistically significant. In this connection we might recall GIAYA's (1920) studies which indicated a rise in the zinc content of certain organs with increasing age.

The corpuscle values in one and the same subject were found to remain at the same level (see Table XI), even when months had elapsed between tests. This constancy is not surprising in view of the observations described later (Chapter VIII) which indicate that the zinc complex in the corpuscles is of an extremely stable nature. Nor does the plasma zinc of healthy persons seem normally to undergo noteworthy changes. — For possible 24-hour-variations in the plasma zinc see Chapter VI.

As the zinc content of the food varies quite considerably, the blood of vegetarians following a diet quite different from the common one might be expected to be affected accordingly. The author has consequently studied persons subsisting for a long period solely on vegetarian food.

In 10 vegetarians (9 men and 1 woman) the mean plasma zinc was 124 ± 4 ($\sigma = 12$), and the mean corpuscular zinc $1\,400 \pm 59$ ($\sigma = 165$), µg %. The plasma zinc is significantly higher than normal (110 ± 2). The corpuscle zinc was somewhat raised, but not, however, to a statistically significant extent.

[1] σ represents, throughout, the standard deviation.

Efforts have been made, where necessary with the help of the haematocrite value, to exclude from the material all persons with anaemia, however mild, and as far as possible all persons with slight infectious conditions. VIKBLADH (1951) has subsequently shown that even a mild infection is accompanied by a fall in the serum zinc value.

The low plasma zinc values in women during pregnancy and at delivery are reviewed in Chapter VI. In children, special conditions seem to prevail for both plasma and corpuscle zinc at birth and during the neonatal period. The plasma zinc in the newborn, for example, is higher than the corresponding normal value for adults. Even higher figures are found in newborn premature infants, and higher still in foetuses (Chapter VII). Apparently, the plasma zinc successively falls as development proceeds, until at some period during infancy it reaches the adult figure.

The corpuscles of the newborn full-term child contain only 1/3 as much zinc as those of the adult, while the corresponding figure for the premature child is hardly more than 1/4. That is to say, the corpuscle zinc is greatly increased — *i.e.* doubled or even trebled — during the first year of life. This may be compared with the slight variations found in the adult corpuscle zinc.

This fact that the corpuscle zinc increases during childhood and the known increase in the activity of the zinc-containing enzyme carbonic anhydrase, prompted a comparison between the two phenomena, and the resulting diagrams (fig. 4) reveal a correlation. While the investigations into this aspect were in progress, VALLEE *et al.* (1949) published results from a similar study involving anaemic patients, on which they found a similar correlation between zinc content and enzyme activity.

The present author has shown, however, that this correlation can be broken in two different ways. By activating the plasma, the enzyme can be stimulated without changing the blood zinc (BERFENSTAM 1949), or — as was shown in the animal tests (Chapter VIII) — the corpuscle zinc can be raised without effect on the activity of the enzyme.

CHAPTER V

Some Aspects of the Zinc Balance in the Body with special References to Infancy

As mentioned in the introduction, the human adult consumes about 10—15 mg zinc daily in his food (McCance & Widdowson 1942). This is a relatively large quantity equal to that of the daily intake of iron. Zinc is present in all foodstuffs, animal and vegetable.

The elimination of zinc from the body has been studied *inter alia* in the zinc balance tests made on adults (McCance & Widdowson 1942), showing that the bulk of the excess metal disappears via the bowels, the urine zinc remaining low even when the zinc dosage is increased. The low urine zinc persisting after intravenous zinc dosage seems to indicate a secretion from the blood to the alimentary canal, probably through the large glands or the intestinal mucosa.

The zinc metabolism has been studied with the aid of radioactive zinc (Sheline *et al.* 1943). The tests showed that zinc introduced into the blood is quickly eliminated from the plasma and absorbed into the liver, pancreas, and kidneys, to be gradually accumulated in erythrocytes and bone tissue.

Many studies on the zinc content of tissues in vertebrates have been published. The results differ very much and the methods used are difficult to evaluate. From recent investigations the following figures, obtained from determinations on dogs, can be given (*cf.* Vallee *et al.* 1949): lungs 10—20 μg/g, pancreas, liver, skeleton, 20—50 μg/g, lymph glands more than 100 μg/g (wet weight).

As already mentioned, zinc deficiency in man is extremely rare, and can only with difficulty be induced in animals, due to the high percentage of the metal in all kinds of food. Figures given for the zinc content of water are 0.03—8 mg/litre (*cf.* Rotz & Weitzel 1919). Data on several foods have also been presented (see Publ. Health Reports, suppl. no. 179) *e.g.* apple 3—9, orange 12, potato 11—14, spinach 67—121 mg/kg dried weight. Of animal materials egg white does not contain measurable amounts of zinc; egg yolk contains 23—29, beef muscle 21—52, mg/kg fresh weight. Excess of zinc in the food is relatively common, due both to extra abundance in certain food, *e.g.* mushrooms, yeast, shellfish, and oysters, and to emanations from cans and cooking

vessels. The amount ingested in one day may therefore be many times greater than usual. If acid foodstuffs have been kept in galvanised vessels, so much zinc may be liberated that the food is positively dangerous (see *Brit. Med. J.*, I: 201, 1923, editorial, DORNICKX 1938, STAS 1938).

Parenteral zinc dosage is also relatively common, *i.e.* in zinc-insulin therapy of diabetic patients.

A supposition has been avoiced that the amount of metal received by a diabetic treated with zinc protamine insulin would in time have a harmful effect on the organism (*cf.* MÖLLERSTRÖM 1943). To the present author's knowledge however, no certain clinical symptoms of a chronic intoxication of this kind have ever been communicated.

A theoretical calculation does not indicate that the amount of zinc administrated to the body in the form of 50 units of zinc-protamine insulin daily for an extended period would be of any importance compared with the zinc available from food.[1] This is also borne out by the results obtained by the present author when determining the plasma and corpuscle zinc of a small material of diabetics, and also by VIKBLADH's investigations (1951) of the serum zinc of similar subjects. It is not definite whether the chronic irritation sometimes arising in tissues round the point of injection can be attributed in any way to a specific action of the zinc in the zinc protamine insulin.

A few experiments are recorded of parenteral zinc dosage of humans. VIKBLADH (1951), for example, has given up to 4 mg zinc as zinc chloride intravenously. VALLEE *et al.* (1949) who found lowered leucocyte zinc in sufferers from leukaemia, dosed these cases with zinc intravenously as a therapeutic attempt, finding thereby that an adult can tolerate an intravenous dose of 2 mg zinc as zinc gluconate per kg body weight. Thus leukaemic patients received repeated doses of 50 mg zinc as zinc gluconate intravenously, up to 300 mg zinc.

Regarding children only few studies have been published. SCOULAR (1939) and STERN *et al.* (1941) have made investigations of pre-school children, showing this age-group retains 0.1—0.2 mg zinc per kg body weight per day. On the other hand no balance studies in infancy have been published, though at this particular period considerable changes take place at least in the blood zinc (BERFENSTAM 1949). Per unit body weight the total zinc con-

[1] The amount of zinc included in a dose of 50 units is calculated at about 0.1 mg, constituting about 1/100 of the zinc customarily taken in. It is plain that no change in the blood zinc can reasonably be expected from such an addition, since it could not alter the plasma zinc more than the almost indiscernible amount of from 100 to 103 μg %, and this if all the zinc could be absorbed instantly.

tent in mammals seems to alter appreciably during life. In investigations of 1921, on rabbits, BERTRAND & VLADESCO have shown an increase during the foetal period with a decrease for the suckling stage, followed by another increase. They attributed the decrease to the small amount of zinc in the mother's milk, and the subsequent increase to the food received after weaning. As in some other of the earlier investigations, it is however difficult to evaluate the reliability of the method used.

An investigation into the zinc metabolism of the infant postulates a knowledge of the *zinc content of its food — i.e.* mother's milk or cow's milk mixtures — and of *the way in which this zinc is utilized.* A number of zinc analyses of milk have already been made *e.g.* by LUTZ (1926), SATO & MURATA (1932), KOGA (1934) and ARCHIBALD (1943), who found the concentration in both human and cow's milk to lie between 300 and 1 000 μg % with the highest values at the beginning of lactation.

Author's Investigations

The author has carried out analyses both on samples of human milk and cow's milk, and also on colostrum, and on modified cow's milk. A few zinc determinations on the faeces of infants were also made.

Methods and material

The same methods could be used in extracting zinc from milk and milk mixtures as were employed in preparing blood samples for analysis, that is treating with strong hydrochloric acid followed by precipitation with trichloroacetic acid, suitability being proved by parallel extraction and ashing processes. The faeces were also prepared in the same way.

The investigated *breast milk* consisted partly of pooled milk from the mother's milk bank, Children's Hospital, Uppsala, partly of random samples taken from nursing mothers. The latter samples were collected directly in zinc-free vessels, and were usually a mixture of two portions taken at the beginning and end of the feed respectively.

The *cow's milk* consisted partly of the commercial pasteurised

commodity, with a standardised fat content of 3 %, partly of milk taken directly from the cow in zinc-free vessels, these samples also being a mixture of portions taken at the beginning and end of the milking.

The *milk mixture* was prepared from a dried-milk preparation "BABY-SEMP," corresponding to a gruel of half cow's milk and half water, with 2 g of wheat flour and 5 g of sugar added to 100 g of the milk mixture, this being the standard artificial food in the Children's Clinic.

Results

The results from parallel extraction and ashing procedures on different kinds of milk are shown in Table XIII, and they indicate a good agreement between the two methods.

The results of the zinc determinations on milk thus obtained, are shown in Table XIV. As appears, the proportions of the metal in breast milk and cow's milk are very similar and in both the concentration is highest in the colostrum. Furthermore samples of human milk taken from the first months of the lactation period show a considerably higher zinc content than those taken at a

Table XIII. *Comparison between ashing in platinum crucibles and extraction with acid as methods for measuring the zinc content of different milks.*

Milk specimen	µg % zinc in the milk specimen	
	Extracted with acid	Ashed
Human milk	522	490
	357	333
	530	600
	160	158
Cow's milk	387	360
Modified cow's milk	308	293
	244	257
	240	250

Table XIV. *The zinc content of human milk, cow's milk, and modified cow's milk.*

	Milk specimen	Zinc content µg %
Human milk	1st day after delivery (colostrum)	2 300
	2nd » » » »	1 080
	» » » » »	1 140
	» » » » »	2 000
	4th » » » »	530
	» » » » »	360
	1st—2nd month after delivery	520
	» » » » »	360
	» » » » »	180
	3 months after delivery	160
	3 » » »	80
	6 » » »	65
	7 » » »	50
	9 » » »	40
	Pooled breast milk I	330
	II	370
	III	390
Cow's milk	1st day after delivery (colostrum)	2 200
	2nd » » » »	2 050
	» » » » »	1 900
	2 weeks after delivery	510
	4 months » »	420
	6 » » »	490
	Commercial milk I	370
	II	440
	III	420
	IV	390
	V	370
Modified cow's milk	I (31.5. 1950)	260
	II (2.8. 1950)	200
	III (12.1. 1951)	310
	IV (22.1. 1951)	240
	V (16.5. 1951)	240

Table XV. *Zinc intake and output in three infants, over a period of one week.*

No. of case	Age (weeks)	Weight at birth g	Weight g	Amount of milk ml	Zink in the milk µg %	Amount of faeces g	Zinc in faeces µg %	Total zinc in food mg	Total zinc in faeces mg
4/51	5	2 500	2 340	3 060	250	69	10 000	7.6	6.9
2/51	3	4 120	3 960	3 950	250	73	11 000	9.9	8.0
7/51	1	2 170	2 150	2 040	250	41	9 800	5.1	4.0

later stage. Samples of the milk mixture, taken at long intervals, showed a remarkably constant zinc content, of the same order of magnitude as that of breast milk during the first three nursing months.

To see how far the infants organism availed itself of the zinc supplied through the milk, a record was kept on a few children of the amount of metal leaving the body in the faeces. The intake of zinc was calculated from the results of the analyses of milk and milk mixture respectively. For results, see Table XV.

Discussion

These investigations show, that relatively large quantities of zinc are conveyed to the infants through the milk. It can be calculated, that a suckling in the 3rd to 4th month who gets 800 g of milk a day receives 1.5 mg zinc daily, while a newborn or premature infant consuming about 300 g daily will receive about 1 mg zinc. The total body zinc of the child in the neonatal period is calculated at about 60 mg (WIDDOWSON & SPRAY 1951) of which the daily addition thus constitutes 1/60. The corresponding value for adults is 1/200. During infancy the supply of zinc appears appreciably to exceed that of iron and copper. According to WALLGREN (1931) the iron content in breast milk is about 50 µg %, and in cow's milk 20—30 µg %. The submitted copper values vary a great deal: from 30 to 100 µg % in breast milk (the last value is for colostrum) and somewhat lower in cow's milk (LESNÉ & BRISKAS 1937, MUNCH-PETERSEN 1950).

Balance tests threw some light on the question of how this large daily consignment of zinc was put to use by the infants. Naturally such tests contain considerable sources of error. It is extremely difficult to collect the faeces in bulk and yet avoid all contact with zinc. Collection was made easier, however, by the fact that the children in the tests suffered from inoperable myelomeningocoele and had the hard stools usual in this condition. To avoid direct contamination by zinc-containing products, no ointments or adhesive tape were used during the tests. Allowances had to be made, of course, for a small amount of zinc leaving the body in the urine, but as any collection in bulk and with the necessary precautions would have involved serious technical difficulties, and as repeated random tests had proved that, as with the adult, the zinc concentration in infants' urine is very low (20—50 μg %), this aspect was omitted in the calculations. In any case during the one week the test lasted, the figure could hardly have amounted to more than a fraction of a milligram.

The author realises, that the above-mentioned sources of error make rigid conclusions difficult. Nevertheless, the figures do seem to show, that *the infants retain very little zinc*, since *the amount given and the amount eliminated are of similar magnitude.*

This result and the above-mentioned finding of a zinc intake which is very large in proportion to the total body zinc, indicates that *there can be no zinc deficit whatsoever during this period.* There must therefore be quite other reasons for the decrease in the zinc content which BERTRAND & VLADESCO found in the young animals during the lactation period.

The tests were continued inasmuch as tolerance to peroral zinc dosage was tried out for one week on the three cases mentioned above. The infants were given a daily dose of 10 or 40 mg zinc in the form of zinc acetate solution mixed with milk. It was found, however, that they tolerated this very well, although the dose corresponded to a quantity many times greater than that normally present in their food.

CHAPTER VI

Blood Zinc during Pregnancy and at Delivery

Several investigators have made detailed studies of the mechanism governing the passage of nutriment from mother to foetus through the placenta. One approach was to observe the changes in the concentration of the respective substances in the blood of pregnant women and in the umbilical cord blood at birth. Fundamental works as regards metals are FLEXNER and co-workers' investigations into the transport of sodium (1948) and iron (1950) through the placenta, made with the help of radioactive isotopes.

In an earlier paper (1950), the present author stated that the plasma zinc of pregnant women is lower than normal and — as far as the state of affairs at delivery is concerned — VIKBLADH subsequently (1951) confirmed this finding.

Material and Methods

In the present investigation, the zinc content in corpuscles and plasma was studied on women at different stages of pregnancy. Samples were also taken from umbilical cord and mother in some 30 deliveries, to compare the blood zinc values in mother and infant.

Most of the samples were taken from healthy pregnant women at the Ante-natal Centre, Obstetric Clinic, Uppsala. We hoped to procure material from all stages of pregnancy, but could naturally obtain only a few samples from women in the three first months, since this category seldom attends the Maternity Centre. In about two thirds of the cases an analysis was made on both whole blood and plasma, and the rest only on plasma.

At delivery the umbilical blood was allowed to run directly from the placental stump into test-tubes containing heparin. Venous puncture of the mother was usually made within one

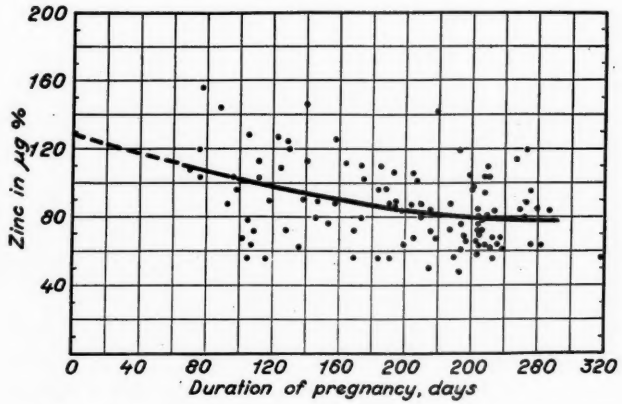

Fig. 5. *Zinc content of plasma in different stages of pregnancy.*

hour after delivery, and never later than two hours from this time. All samples were taken from healthy mothers delivered at the Obstetric Clinic, Uppsala.

Results

The plasma zinc content of 106 pregnant women has been examined. The determinations were made at different periods during pregnancy after the tenth week. The values obtained are shown in Fig. 5. The normal series reviewed in Chap. IV was used for comparison; this should be a suitable choice, since it consisted entirely of women of child-bearing age. The mean for plasma in this latter material was 108 ± 4 µg %. For the 106 pregnant women it is 86 ± 2 µg %, with a standard deviation of 23 µg %. This value is significantly lower than that for non-pregnant women. A division into 4 groups according to the point of pregnancy at which the zinc analysis was made gives the means shown in Table XVI.

A significant decrease is seen to take place during pregnancy, the difference between the means for the first and the last stage being 18.9 ± 5.9 µg %. It is impossible to say definitely whether the decrease is successive throughout pregnancy; it seems, how-

Table XVI. *The zinc content of plasma during pregnancy. A series of 106 women divided into four groups according to the stage of pregnancy.*

Duration of pregnancy	No. of women	Mean ± standard error µg %
70—160 days	30	99.2 ± 5.1
160—200 »	18	86.6 ± 4.4
200—240 »	22	80.7 ± 4.9
240—280 »	34	80.3 ± 2.9

ever, as if it was in the main completed about the 30th week, after which any further fall must be relatively slight. This is also borne out by Fig. 5, where the curved line best follows the observed value of plasma zinc. This curved line, obtained with the method of least squares, expresses the general trend in these zinc determinations, though there may be several cases of individual deviations.[1] The lack of values for the zinc content during the first 70 days of pregnancy naturally confines the validity of the curve to the 10th—40th week.

No difference in plasma zinc was found between primi- and multiparae. In 33 primiparae the mean was 82.0 ± 3.2 µg % and in 56 multiparae 84.9 ± 3.2 µg %. The difference is thus 2.9 ± 4.6 µg % and is not statistically significant.

In 67 of the above 106 women, the *zinc in the corpuscles* was determined at the same time as the plasma zinc. The mean was 1 267 ± 27 µg %, and the standard deviation 220 µg %. The corresponding figure for 42 non-pregnant women was 1 202 ± 30 µg % (Table IX), so that the difference between the two of 65 ± 40 µg % is not statistically significant. If, however, the material is again grouped according to the moment at which the determination

[1] The statistical calculations show that a straight or a curved line fits the observed values equally well. The quadratic parabola $y = 134.5 - 0.39\,x + 0.00067\,x^2 \pm 21.4$ gives a somewhat smaller standard deviation round the fitted curve than the straight line $y = 114.0 - 0.139\,x \pm 21.8$. In the equations, y denotes the expected value of µg % Zn and x the number of days of pregnancy.

Table XVII. *The zinc content of blood corpuscles during pregnancy. A series of 67 women divided into three groups according to the stage of pregnancy.*

Duration of pregnancy	No. of women	Zinc content in corpuscles µg %
70—200 days	22	1 121 ± 39
200—240 »	18	1 347 ± 47
240—280 »	27	1 333 ± 40
70—280 »	67	1 267 ± 27

was made, it is found that the zinc in the corpuscles rises during pregnancy (Table XVII), the means increasing from about 1 100 µg % for the earlier period to over 1 300 µg % for the later ones. The differences are 226 ± 62 µg % and 212 ± 56 µg % respectively and are therefore both statistically significant. The author has thus been able to show that the plasma zinc falls and the corpuscle zinc rises during pregnancy.

The results of the investigations into the behaviour of zinc on both sides of the placenta at delivery are most clearly shown in Fig. 6. The plasma zinc in the blood of umbilical cord was considerably higher (121 ± 6.4 µg %) than in the mother's venous blood (85 ± 4 µg %). It was equal to, or lower, than that in the plasma of the mother in only three cases. There is also a statistically significant positive correlation between the respective plasma zinc of mother and child at delivery. On a material of 28 mothers and children, the correlation coefficient was $+0.51 \pm 0.11$.[1] This means, that a high value in the mother is often accompanied by a high value in the infant.

As Table XII shows, the zinc in the corpuscles of the umbilical cord blood is several times lower than in the venous blood of the mother.

[1] According to the Bravais-Pearson formula, *viz.* $r = \frac{\Sigma x y}{n \cdot \sigma_x \cdot \sigma_y}$, where x and y denotes the deviation from the resp. means, and σ_x, σ_y the resp. standard deviations.

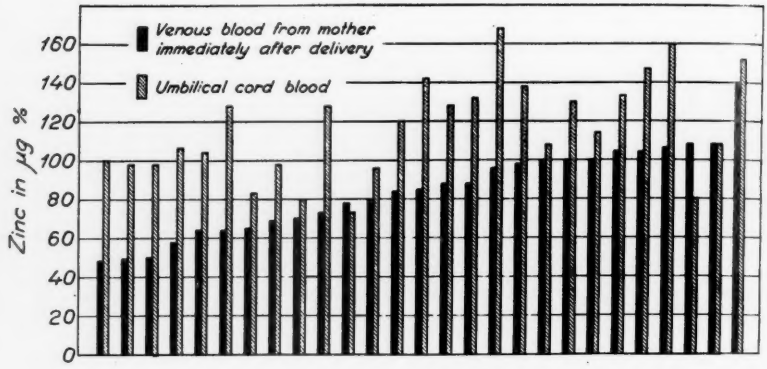

Fig. 6. *The zinc content of plasma from mother, and from umbilical cord, at delivery.*

Discussion

It would naturally be very enlightening if samples for zinc analysis could be obtained from the same woman before, during, and after pregnancy. This, however, has not been possible, and the material had to be compiled from samples from a large number of women at different stages of pregnancy.

During pregnancy plasma zinc is thus subject to a fall, which does not seem complete till some months before delivery, when the value is about 3/4 that of the normal.

Contrary to the normal series the samples from pregnant women are all taken in the afternoon, as the Ante-natal Centre has its consultation time between 2 p.m. and 4 p.m. Knowing, that there is a 24 hour-rhythm in the serum iron, it would here be of importance to know if the zinc content of the plasma was also subject to the same regular fluctuations. WOLFF (1951), studying this problem, has not been able to find any hard-and-fast rule in this connection, but VIKBLADH (1951) has found 8 % lower plasma zinc values in tests in the afternoon than in morning samples. By comparing the results from plasma zinc determination in pregnant women we must bear this fact in mind, the decrease in the plasma zinc is however so pronounced (about 20—25 %) that it cannot conceivably be attributed to rhythmic changes.

The slight advance which the maternal plasma zinc immediately after delivery shows over the concentration during the last part of pregnancy, falls within the limits of the standard deviation. But it is also quite possible that the change would have had time to take place in the hour or so following birth. Here the samples were taken at all times of the night and day — though chiefly during the night and in the morning — so that the influence of a possible 24-hour rhythm cannot be totally ruled out.

It must be remembered that during pregnancy there is a successive increase of the total plasma volume, generally interpreted as hydraemia (for references see LUNDSTRÖM 1949). Probably the effect of this hydraemia must be taken into consideration when judging the plasma zinc values of pregnant women.

It was already known that the iron and copper in the serum underwent changes during pregnancy, and it will not be out of place to dwell a little on these phenomena, for purposes of comparison. Thus, there is a fall in the mother's *serum iron* during pregnancy, but only from the second quarter on. During the last three months it is so great that, at delivery the concentration is not more than about 2/3 of the normal (see LUNDSTRÖM 1949). The serum iron of the newborn infant on the other hand, is raised (VAHLQUIST 1941). Contrariwise, *serum copper* rises during pregnancy and may at the end often be double the normal figure. The rise is already perceptible during the first months. Again contrary to serum iron, the serum copper on the foetal side of the placenta is low (*i.a.* HOLMBERG 1941).

It is noteworthy that both the fall in serum iron and the rise in serum copper of the mother has been attributed to the foetus's need for relatively large quantities of metal. It has been suggested, that the high copper serum value in the mother indicates constant large reserves of this metal to meet the requirements of the foetus, which then stores it in the liver. On the other hand, the low serum iron in pregnant women has been taken to indicate that the metal is drawn from mother to foetus.

While it is *in utero*, the foetus stores up about 60 mg of zinc (WIDDOWSON & SPRAY 1951). As in the study of the passage

of other metals through the placenta, the question again arises whether this zinc is taken from the plasma or the corpuscles of the mother. The results obtained in the present investigation suggest the former source.

The chief support for this theory was found in the intravenous zinc-tolerance tests on rabbits undergoing caesarean section (see Chapter VIII). It was there possible to show that an experimentally induced strong increase in the mother's plasma zinc was rapidly followed by a rise in the plasma zinc of the young. A proof that the serum iron of the foetus is, similarly, supplied by the mother was provided by the tests of POMMERENCKE *et al.* (1942) with radio-active iron, where this metal was recovered from the foetal plasma less than an hour after administration to the mother.

It has furthermore been found that there is a positive correlation between the zinc content in the plasma of mother and foetus respectively — that is to say, a high zinc content in the mother is followed by high zinc content in the foetus, and conversely, low maternal values give low foetal values. This must be taken to indicate that the plasma zinc of the foetus is directly dependent on that of the mother.

Nor is a newborn infant's total zinc — 60 mg — too much to have come from the mother's plasma, particularly as it seems established that this same plasma supplies the foetus with all its 300 mg of iron (VOSBURGH & FLEXNER 1950). And, as already mentioned, the iron and zinc are present in the plasma in about the same concentration.

There is, however, no reason to suppose that the foetus's very small daily requirements of zinc — during the last month 0.5 mg per day at most — would upset the mother's zinc balance by a lowering of the concentration in the plasma, particularly as she is known to consume each day more than 20 times the amount assimilated by the foetus (MCCANCE *et al.* 1942). Another proof of this is that the fall in the mother's plasma value is noticeable as early as the 3rd—4th month, when the needs of the foetus are certainly very low, perhaps 0.1 mg zinc per day.

The statistical treatment of the material has shown the low

plasma zinc value during the last part of pregnancy to be accompanied by a statistically significant increase in the blood corpuscle zinc. This suggests a negative correlation between the zinc content of these two components, and a calculation of the correlation coefficient proves this to be the case (-0.39 ± 0.10). This rise in the corpuscle zinc is difficult to explain. We might, in this connection, mention WOLFF's (1951) observations of a raised corpuscle zinc in certain pathological conditions accompanied by low plasma zinc. And it may finally be pointed out that the above-mentioned negative correlation cannot be demonstrated in men and non-pregnant women.

The observations of low zinc content in the blood corpuscles of the umbilical cord blood are in good agreement with the findings in newborn infants (Chapter IV). As in the latter group, the blood of the umbilical cord also shows a carbonic anhydrase activity which is low compared to that in the adult.

CHAPTER VII

The Zinc Content in Foetal Blood

The present investigation has shown that the corpuscle zinc of full-term newborn infants is considerably lower than it is in the adult, while that of the premature newborn infant is even lower; and further, that the plasma zinc also undergoes certain changes with age. It therefore seemed that the material could be profitably expanded by a study of the zinc in the blood of foetuses.

Material

Samples for analysis were taken from foetuses obtained from surgical abortions in the 4th to 6th month of pregnancy, carried out at the Gynaecology Clinic, Uppsala. Only specimens from abdominal caesarean sections could be used. In none of the cases was the woman suffering from any somatic disease.

Blood from the foetus was obtained from the umbilical cord, great care being taken to avoid haemolysis. Venous blood from the woman was in all cases collected within 2 hours of the removal of the foetus.

Results

The mean plasma zinc in 10 foetuses (100—150 days) was $300 \pm 31\,\mu g\,\%$ ($\sigma = 99$). This value is almost three times that of the corresponding adult level, and is also considerably higher than the values in premature infants ($187 \pm 20\,\mu g\,\%$) and the newborn ($125 \pm 5\,\mu g\,\%$). The differences are 113 ± 37, and $175 \pm 32\,\mu g\,\%$, and are thus statistically significant. *The corpuscle zinc* in foetuses was $344 \pm 20\,\mu g\,\%$ ($\sigma = 60$), and so can be said to correspond with the value in premature infants (347 ± 13), and to be only slightly less than that in the new-born ($376 \pm 19\,\mu g\,\%$). Table XVIII.

Table XVIII. *The zinc content of plasma and corpuscles in mother and foetus in surgical abortions between the 4th and 6th months of pregnancy.*

Date	No. of case	Name	Duration of pregnancy days	Haematocrit value	Zinc in plasma µg %	Zinc in corpuscles µg %	Length of foetus cm	Haematocrit value	Zinc in plasma µg %	Zinc in corpuscles µg %
19.9. 50	1 789/50	I. V.	150	37	222	1 940	33	43	240	350
29.11.	2 295/50	E. B.	129	42	124	1 120	20	33	112	290
14.2. 51	318/51	B. A.	119				23	41	350	320
15.2.	304/51	G. P.	112	39	113	1 000	20	33	305	385
18.4.	780/51	R. E.	118	44	90	1 560	20	37	320	300
16.5.	993/51	H. S.	105	38	78	920	15		290	
26.6.	—	W.	150		100		25	39	275	345
5.7.	1 267/51	M. K.	154		76		24	43	230	325
26.10.	1 989/51	H. L.	130	37	120	810	22	32	475	475
18.12.	2 493/51	G. E.	131				23	41	400	310

Discussion

Needless to say, only very small samples could be obtained from the foetuses, *i.e.* 1—4 ml. Certain analyses had to be made on plasma volumes of no more than $^1/_2$ ml. The results must therefore be presumed less trustworthy here than in the rest of the investigation.

The material is too small to allow any definite conclusions as to the changes in plasma and corpuscle zinc during different months of pregnancy. However, even in the 4th month, the *corpuscle values* seem to have reached a level not far below that of the newborn infant. The plasma zinc, on the other hand, shows values higher than any yet found in any condition, either normal or pathological, during the whole course of life. It has already been mentioned that the plasma zinc values of premature newborn infants are higher than those of children born at term, and we can therefore point to a successive fall as the foetus develops.

A comparison between the plasma zinc and the plasma iron

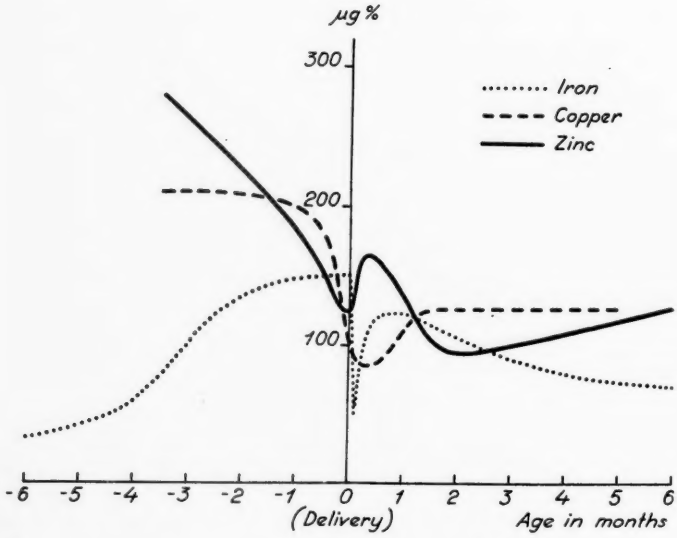

Fig. 7. *Schematic representation of the variations in the serum content of iron, copper, and zinc during foetal life, at delivery, and during the first six months of life.*

Figures for iron from VAHLQUIST (1944), for copper from LESNÉ, ZIZINE & BRISKAS (1936).

and copper during the foetal period and the first part of infancy respectively is shown in Fig. 7. Serum iron is seen to be low right up to the 5th—6th month of pregnancy; it then rises, to reach its maximum (about 160 µg %) at delivery, after which it (VAHLQUIST 1941) falls during the first year of life to about 60 µg %. Serum copper, on the other hand, is high during the first months of pregnancy and falls, during the last quarter, to its lowest value (less than 100 µg %) at delivery, then adjusting itself to a slightly higher level during the first year of life (LESNÉ *et al.* 1936).

As can be seen, there is a great difference between the low plasma zinc values of the mothers and the high ones of the foetuses. It has not in this material been possible to show that a relatively

higher plasma zinc in the mother has a similar response in the foetus, as is the case at delivery (Chapter VI).

The high zinc content in the foetal plasma, especially during the first few months, should be able to provide more than enough metal for building up the zinc protein in the corpuscles. The fact that corpuscle zinc rises so slowly would suggest that this building up depends on some other factor then the zinc supply in plasma.

Our findings show that, during the whole of pregnancy, the placenta is the scene of an active secretion process providing the transport of metal from mother to foetus, since the concentration of zinc in the foetal plasma is always higher than that of the mother. As far as zinc is concerned, then, there is no question of a simple dialysis in the placenta during any part of pregnancy. Iron, on the other hand, may possibly be transferred from mother to foetus by dialysis in the placenta during the first 7 months of pregnancy. Only after this period does the child's serum iron value begin to exceed the mother's, so that from this juncture, at any rate, some active process in the placenta must be presumed to take place.

It has previously been shown (BERFENSTAM 1952) that there is a correlation between corpuscle zinc and the activity of the enzyme carbonic anhydrase in the blood. The newborn, and particularly the prematurely born, have a very low enzyme activity, corresponding to the low corpuscle zinc. In foetuses in the 4th to 6th month of gestation the zinc content, as mentioned above, differs only very slightly from that found in premature infants; on the other hand, as was shown in an earlier investigation (BERFENSTAM 1952), foetuses at this stage of development sometimes show no enzymic activity whatsoever in their blood.

CHAPTER VIII

Experiments on Animals

Some experiments with zinc dosage of man and animals have been made previously[1], though not in order to study possible changes in the zinc content of *the blood corpuscles*. The present author's intention has been to study such possible changes in corpuscles and plasma after administering zinc; and, should such occur, to note at the same time the activity of the zinc-containing enzyme carbonic anhydrase, having demonstrated that there is normally a certain relation between these two factors.

Special interest centred round tests to be made during pregnancy and the neonatal period, these being times when, as has been shown in the human subject, the blood zinc has quite other levels than later in life.

The problems can be summed up thus:

1. Is it possible to influence the corpuscle zinc in the adult by means of zinc administration? (This involves a study of the effects upon plasma and blood corpuscles of single, and of repeated, intravenous and oral zinc dosage.)

2. Is it possible to influence the corpuscle zinc in the foetus and the new-born by administration of zinc to the mother during pregnancy?

3. Is it possible, by administration of zinc to the mother during lactation, to accelerate the increase in the corpuscle zinc during the neo-natal period?

4. If an increase in the corpuscular zinc can be brought about, is it also possible to influence simultaneously the activity of carbonic anhydrase?

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[1] For references see Vallee B. L. & Altschule M. D. Physiol. Rev. *29*: 370, 1949.

Now, we still did not know what zinc compounds would be suitable for intravenous or peroral administration, nor what risks such dosage might incur. It is true that this metal had been given parenterally in the form of zinc protamine insulin in diabetes therapy; but, even with a high dosage of insulin, the quantities of zinc injected are so small that they cannot affect the plasma zinc at all. (Chapter V.) McCance & Widdowson (1942) and Vallee (1949) have given zinc salt solution intravenously to a few experimental subjects, and Vikbladh (1951) has studied the change in the serum zinc after injections of zinc as zinc chloride solution in man.

Apart from reports of official investigations into the occupational disease known as zinc or metal fume fever, caused by inhaling zinc fumes (*cf.* Public Health Reports, suppl. No. 179), the literature contains little on the toxicity of the metal. The details available are based on single cases of poisoning after the consumption of zinc salts, and the exact size of the dose is usually not given. From the information available it was therefore difficult to decide how much zinc could safely be given in the projected tests without producing toxic side-effects.

It was obviously too risky to experiment in this way on the human subject, particularly during pregnancy, so that animals had to be used, in the first place. Rabbits proved suitable subjects for the purpose: it was easy to obtain blood for analysis both from the adult and from the newborn animals. And, as in the human subject, the zinc content in the corpuscles of the young was found to differ appreciably from that of the adults.

The literature provides few details of zinc tolerance in animals. The lethal dose is, according to Schwartze & Alsberg (1923), 435—500 mg zinc (as zinc sulphate)/kg body-weight given by mouth, and 10 mg zinc/kg body-weight intravenously. According to Vallee (1949) dogs tolerate 2 mg zinc (as zinc gluconate)/kg body-weight intravenously without toxic symptoms. Drinker *et al.* (1927), who made long-term tests on cats and dogs, gave 175—1 000 mg zinc oxide daily for periods of from 3 to 53 weeks and no abnormalities were observed. This refuted the earlier view that prolonged dosage of zinc caused anaemia.

Plan of the investigation

Zinc was given to the laboratory animals intravenously and by mouth. The effect on the serum zinc and the corpuscles was studied both after one single dose and after repeated dosage. Zinc was also given to pregnant rabbits by injection and by mouth, after which observations were also made of the litter's blood zinc.

Material and methods

The animals were bred at the Department of Pathology, Uppsala, and fed on the basic diet usually given there (about 200 g grain, 200 g hay and 50 g Swedish turnip per rabbit per day). Fairly young but fully adult animals were used for the experiments, but no consideration was taken of colour or race. Unless otherwise stated, the animals weighed about 3 kg.

For technical reasons we chose to give the extra zinc in the form of a salt of a weak acid, and zinc acetate was chosen. To keep the zinc concentration constant, the salt solution must be adjusted to an acid pH value (pH 3—4). For peroral dosage a solution containing 50 mg Zn per ml was used, and for the injections, solutions in concentrations from 1 to 10 mg Zn per ml. In these cases, the solutions were rendered isotonic with sodium chloride.

The *intravenous* dosage was performed in the usual way by injection into an ear vein. Usually, a strength of solution was chosen to allow about 5 ml to be injected. The operation was done slowly. The *peroral* dosage was at first performed with a tube, but after some weeks the animals tended to object to this method. This meant that the solution might get into the bronchial tree, resulting in abscesses in the lungs. It was found simpler to get the solution down with the help of a syringe, whose large, bulbous tip was placed at the root of the tongue. While being fed in this way, the rabbits were kept upright, and therefore could not help swallowing the solution.

At first, blood samples were taken from the ear vein into a glass vessel attached to a vacuum pump. The ear was first washed with zinc-free alcohol, and the blood then ran directly down into

the tube containing dry heparin. Rubber tubing and stoppers were avoided, ground joints of Pyrex glass being used instead. In the intravenously injected cases, the greatest care was necessary to prevent contamination of the blood samples by zinc solution. The injected ear was therefore immediately bandaged, and the samples always taken from the other one. Rabbits receiving the zinc by mouth were isolated as far as possible, to prevent them coming into contact with one another and getting their fur soiled with zinc solution. During the greater part of the investigation, however, the samples were taken by cardiac puncture with glass syringes washed in acid. This lessened the risk of contamination considerably.

In the case of the young rabbits, samples were always taken by cardiac puncture. As a rule, in newborn rabbits, samples were taken at each test from two animals from the same litter, about 1—2 ml blood from each. The corpuscle samples were usually analysed separately, while the plasma samples often had to be made into one to provide a sufficient quantity for analysis, since the amount obtained from, say, 1 ml blood with a usually high haematocrit value was too small for a reliable result. (The control investigation proved this to be permissible in the case of newborn animals, since all the members of a litter had about the same zinc content in both plasma and corpuscles, as will be shown later). Except for the newborn, most of the animals survived the puncture. Samples were usually taken during the first day of life, and subsequently at intervals of 1—2 weeks up to the age of 5 weeks, when the animals had ceased to suck. To complete the tests, therefore, it was necessary to have large litters, since undue venesection of the same young animal might well upset the formation of its blood.

A. Normal Values of Zinc in Plasma and Corpuscles in Adult and Young Rabbits

The author's values for the normal plasma and corpuscular zinc in newborn and adult rabbits are shown in Table XIX. The mean plasma zinc values in dams at delivery have also been included here, and these are discussed later.

Table XIX. *The zinc content of plasma and blood corpuscles in rabbits.*

Mean of adult rabbits, dams at delivery, and newborn rabbits.

	Plasma			Blood corpuscles		
	No. of animals	Mean ± standard error µg %	Standard deviation µg %	No. of animals	Mean ± standard error µg %	Standard deviation µg %
Adult rabbits	36	248 ± 10	62	36	902 ± 22	134
Dams at delivery.	9	179 ± 16	48	—	—	—
Newborn rabbits .	27	389 ± 13	68	19	528 ± 27	119

It can be seen from the table that the newborn rabbits have in general a higher *plasma zinc* than the adult rabbits. The difference is 141 ± 17 µg%, and is thus statistically significant. The *corpuscle zinc* is, on the other hand, considerably lower in the young than in the adult rabbits. The difference is 374 ± 35 µg%. The corpuscle zinc in the newborn is thus only about half that in the adult rabbits. The increase takes place relatively quickly, as can be seen in the diagram (Fig. 13) and at the age of 3–4 weeks the young have, in general, a corpuscle zinc level corresponding to that of the adult.

B. The Plasma Zinc after Administration of Zinc to Adult Rabbits

a. Plasma zinc in adult rabbits after intravenous zinc dosage

When samples for analysis are taken immediately after intravenous injection of zinc salt (*i.e.* within 3 minutes) a considerable rise is, as expected, found in the zinc content of the plasma. A dose of, say, 10 mg to a rabbit of normal body weight gives a plasma zinc value of about 5000 µg%. The plasma zinc curve falls steeply during the first few hours, but is then levelling off. After administering small amounts (up to 1 mg), the zinc content usually returns to normal in about 8 hours. After large doses, on

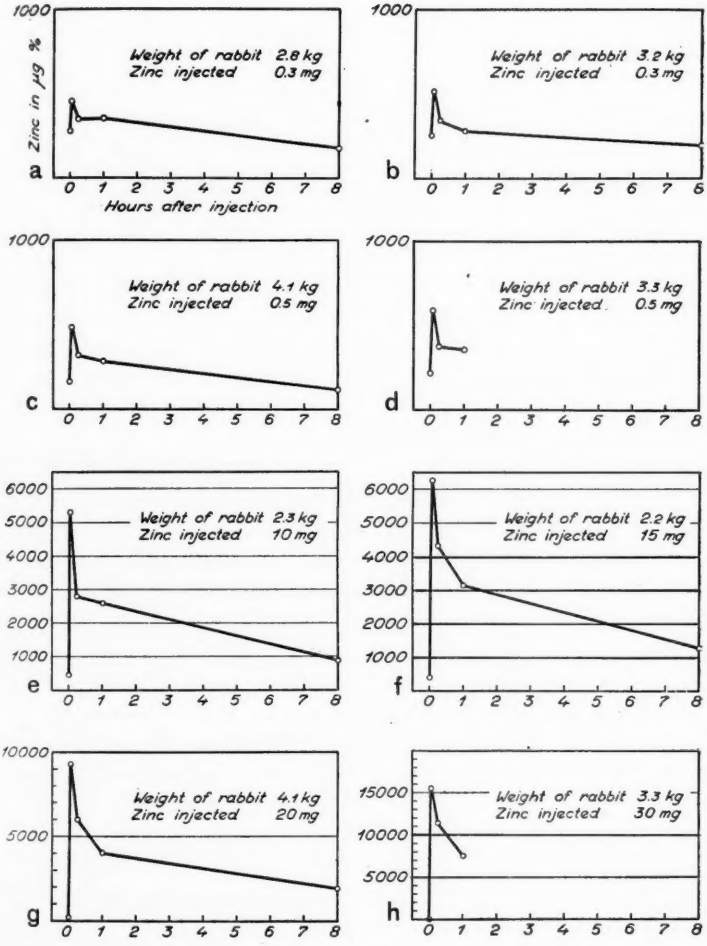

Fig. 8. *The zinc content of plasma in rabbits after intravenous injection of different quantities of zinc as zinc acetate.*

the other hand, the figure may still be above normal after about 24 hours. The course of the curves after administering varying quantities of zinc is shown by the diagrams in Figs. 8 and 10.

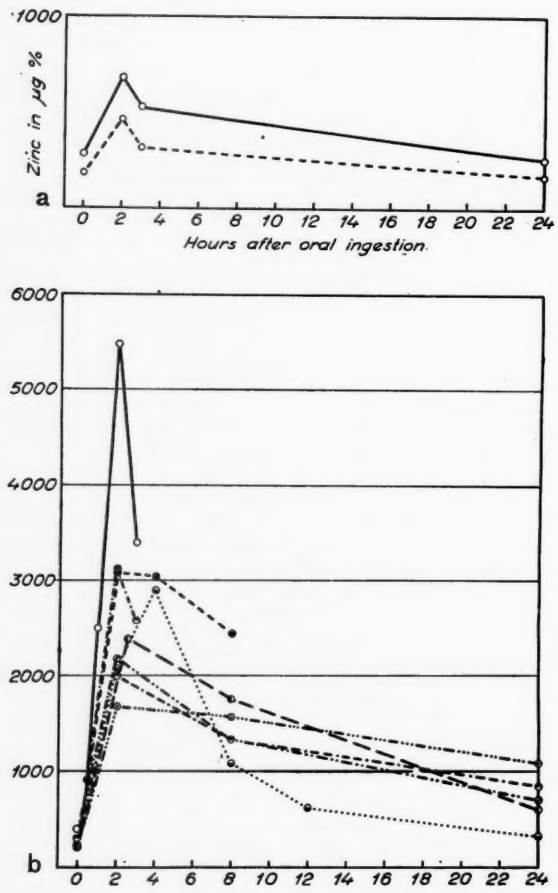

Fig. 9. *The zinc content of plasma in rabbits after oral ingestion of zinc as zinc acetate.*
Doses: a) 100 mg, b) 250 mg.

b. Plasma zinc after zinc dosage by mouth

Peroral zinc dosage also gives a rise in the plasma zinc. The curve reaches a maximum after $1\,^1/_2$—3 hours, and then gradually falls. A dose of 250 mg zinc to a rabbit of normal size, for example,

usually results in a rise of 2 000—3 000 µg % and the plasma zinc curve does not reach the original value until after 12—24 hours. The diagrams in Fig. 9 show the course of the plasma zinc curves after peroral dosage of varying amounts of zinc.

c. Plasma zinc in adult rabbits after prolonged peroral zinc dosage

As peroral dosage was found to give good absorption with subsequent increase in the plasma zinc, this method of administration was employed in tests over a longer period.

The following tests were made on three adult rabbits, to study the behaviour of the plasma zinc in prolonged dosage:

1. Determination of the initial values of plasma and corpuscle zinc.

2. Determination of the zinc content 8 and 24 hours after one peroral dose of 250 mg zinc.

3. Daily dose of 250 mg zinc, and measurement of the zinc content 8 and 24 hours after the 8th, 15th and 22nd dose.

The values in Table XX show that daily peroral zinc doses alter the course of the plasma zinc curve: after the initial rise the curve does not immediately return to normal but remains at a high level. The same is true of the experiments with prolonged peroral dosage made to study the effect of additional zinc on the corpuscles. The sample dated 22.4.1950 was thus taken during prolonged uninterrupted dosage (Table XXI).

Discussion

The normal value of the zinc content in the *plasma* of the adult rabbits is considerably higher than in man. Further, the variations in the plasma values of the rabbits are greater than those shown in man. In these respects the behaviour is similar to that of the serum iron in rabbits, which shows a higher figure and greater individual variations than it does in man.

The *corpuscle* zinc, on the other hand, is somewhat lower in the rabbit than in man. Nevertheless, repeated analyses of the same animal at intervals of several months show that the cor-

Table XX. *The zinc content of plasma and blood*

Tests have been carried out at intervals of one week.

No. of case	Initial		Zinc content					
			First test				Second	
			8 hours		24 hours		8 hours	
	plasma	b.c.	plasma	b.c.	plasma	b.c.	plasma	b.c.
1	285	800	400	900		1 000	680	1 290
2	280	710	640	950	260	850	1 020	1 360
3	134	760	390	980	240	860	3 840	

puscle value remains relatively constant. The material used here is too small to deduce any variations in corpuscle and plasma zinc due to sex and age of the subjects.

The state of affairs in the newborn rabbits reflects that yielded by the investigations on man, *i.e.* a somewhat higher plasma zinc value and a considerably lower corpuscle value than in the mother. The present author has noted another situation in a preliminary investigation of the zinc content of the blood of guinea-pigs, where the difference between newborn and adult seems to be slight — due, perhaps, to the exceptionally long gestation period of this animal in proportion to its size.

The results of the experiments with intravenous zinc dosage show *inter alia* that the strong initial increase in the plasma zinc value bears a certain relation to the amount of metal injected. The highest plasma zinc concentrations, 15 000—20 000 μg %, were noted after an intravenous dose of 20—30 mg zinc.

It would be of interest to know how much of the metal from an intravenous zinc salt injection actually appears in the plasma, since some of it might be immediately lost into the extracellular fluid, or fixed in the reticuloendothelial system, and thus never be bound in the plasma at all. This question can be answered if samples for analysis are taken very quickly after the injection. In this investigation, such samples were taken within 3 minutes, whereby it was found that, if large quantities of zinc had been

corpuscles in rabbits after prolonged repeated dosage.

Samples taken 8 and 24 hours after oral administration.

of blood µg %									
test		Third test				Fourth test			
24 hours		8 hours		24 hours		8 hours		24 hours	
plasma	b.c.	plasma	b.c.	plasma	b.c.	plasma	b.c.	plasma	b.c.
320	830	1 720		900	1 700	4 400	4 900	4 600	6 600
600	1 390	1 020	1 670	950	1 240	1 760	1 670	1 480	1 400
1 410		2 880	5 600	1 440	4 130				

given (*i.e.* more than 10 mg), 50—80 % was recovered, the corresponding figure for small doses (0.3 to 0.5 mg) being about 100 %. In these calculations, the amount of plasma was taken to be 4.5 % of the body weight.

A certain measure of the speed with which the metal disappears from the plasma can be obtained from similar calculations made, say, one hour after the injection. After the larger doses the plasma contains about 30 %; after the smaller, about 70 %.

The results of the *peroral* tests show that it is possible to produce a considerable rise in the plasma zinc value by this route also. Here, too, the increase naturally depends on the size of the dose. Ideally, blood samples should be taken, say, every 15th minute during the 2nd and 3rd hour following the peroral dose, in order to hit the peak of the curve. To spare the animals, however, and to avoid the almost inevitable dilution such frequent sample-taking would involve, we had to confine ourselves to an hourly sample. It is therefore probable that, in most cases, the peak of the plasma zinc curve was missed.

As the diagram shows, animals who received the same zinc dosage show quite appreciable variations in their plasma zinc curves. Many factors may contribute to this besides the varying body weight of the animals. As indicated above, the peak point may well have been missed in many cases. Moreover, the amount of food in the stomach at the time of the reading undoubtedly

Table XXI. *The zinc content of plasma and corpuscles in rabbits receiving zinc by mouth daily.*

Rabbits no. 6 and 8 received daily, for 14 months (April 13th 1950—June 18th 1951) 250 mg. zinc by mouth, with the exception of the four days preceding the taking of each sample.

Date	Rabbit no. 6			Rabbit no. 8		
	Haemato-crit value	Zinc in plasma µg %	Zinc in blood corpuscles µg %	Haemato-crit value	Zinc in plasma µg %	Zinc in blood corpuscles µg %
12.4. 1950	49	312	820	38	290	800
22.4.[1]	44	1 600	2 050	40	2 640	3 500
2.6.	43	306	1 240	40	289	1 220
25.7.	37	430	1 100	40	410	1 200
11.10.	42	359	668	37	423	—
6.11.	41	470	970			
16.11.				37	656	1 200
22.11.	40	300	950			
2.2. 1951	40	—	680	37	—	860
31.5.	39	—	1 020			
22.6.				35	400	890
8.12.				36	270	1 100

[1] No interruption in administration before sampling.

affects the result. And finally, it is always possible that the zinc administered was not always swallowed *in toto*.

To achieve a certain rise in the plasma zinc content, the dose must clearly be many times greater when given perorally than when given intravenously. For example, a peroral dose of 250 mg is found to give about the same rise as that yielded by 5 mg given intravenously. Probably the absorption from the alimentary canal proceeds only successively, allowing time for the elimination of the zinc already bound in the plasma, and, moreover, some of the zinc absorbed may be retained in the liver. However, the conclusion seems warranted, from the great difference observed, that only part of the zinc administered is absorbed from the alimentary canal.

Special conditions result from *daily peroral zinc dosage:* the plasma zinc curve, which ordinarily returns to the initial value

after 12—24 hours, now does not return to this value. The peak seems instead to get higher and higher, and the downward course more and more gradual. When the zinc has been given over a period of weeks, it takes 3 to 4 days before the plasma value approaches the normal level. The reason why the zinc reaches these high levels during prolonged administration can be explained on the assumption that initially it is stored in depots which ultimately become saturated, and are thus no longer able to regulate the zinc content of the blood.

The quantities of zinc used in the experiments may look large compared to the amount presumably included in the normal diet of the animals. But the high doses have been chosen on purpose to make any results significant. Rabbits also seem to have a high zinc tolerance, risk to the animals entering only at doses of 30 mg intravenously or 1 g perorally (corresponding to about 10 and 300 mg per kg body weight respectively). Doses of this size were given in some tests, the plasma zinc values then rising towards 20 000 µg %, and the animals dying about one hour after the injection, or 2—3 hours after the peroral dosage. No special poisoning symptoms in the form of convulsions and the like were observed.

In the longest tests (April 1950—June 1951), (Table XXI) the total amount of zinc salt given to the animals corresponded to a quantity of about 100 g pure zinc. As a rule, the rabbits showed no signs of disease during medication beyond a certain loss of appetite over short periods. Nor did the furs alter noticeably. Beyond the zinc determinations the only other blood tests made were determinations of the haematocrit values, in which, however, no definite change could be observed. Investigation of the subsequently killed animals revealed no *macroscopic* change in the parenchymatous organs. No histological investigation was made.

It is interesting to compare these results with those obtained from similar tests with iron. The first point is that rabbits have a far greater tolerance for zinc than for iron — NEANDER & VAHLQUIST (1949), who studied the toxicity of the latter metal in intravenous dosage, found that the effect varied, but that toxic symptoms in the form of convulsions often appeared when the animals had received no more than 1—2 mg iron per kg body weight.

These two authors also made a closer investigation of the serum iron curve after large doses of iron. They showed that less than 25 % of the iron given intravenously as inorganic iron salt was found in the serum immediately after the injection; 4—5 hours after this point, the serum iron value was approaching the normal level. The corresponding percentage for a large dose of zinc, on the other hand, was 50—80, and the rise in the plasma value lasted considerably longer. Small doses of zinc were maintained even longer in the plasma, which was not really surprising.

These experiments show clearly the great difference in the serum's capacity to bind iron and zinc respectively (*cf.* Chapter III). The reason for the invariably low figures and rapid return to normal in the case of iron seems to be that the iron-binding fraction of the serum has a certain saturation limit which, at all events in man, is about 3—400 μg % (LAURELL 1947). Excess iron is rapidly eliminated. Within the limits dealt with here, zinc probably has *no* saturation limit: more of it can therefore be bound in the plasma, and the high plasma zinc values are thus maintained for a number of hours. This strongly suggests that the zinc is actually bound in a high-molecular complex in the plasma. Dialysis at a neutral pH of plasma enriched *in vivo* also confirms that all the metal is present in an undialysable form. This tallies with the dialysis of similarly zinc-enriched plasma *in vitro*, where the zinc concentration may easily reach values of the same size as in *tolerance tests*, the metal still remaining on the whole undialysable.

C. Effect of Raised Plasma Zinc on the Corpusle Zinc in Adult Rabbits

Bearing in mind that high levels of plasma zinc can be obtained following intravenous or oral administration of zinc, it was thought that the corpuscle zinc might also be influenced in this way. A study also revaled that, when the plasma zinc reaches a certain level, it does cause a rise in the zinc content of the corpuscles (see Figs. 10 and 11). As appears from the diagram, the — relatively moderate — rise in the corpuscle zinc curve occurs later

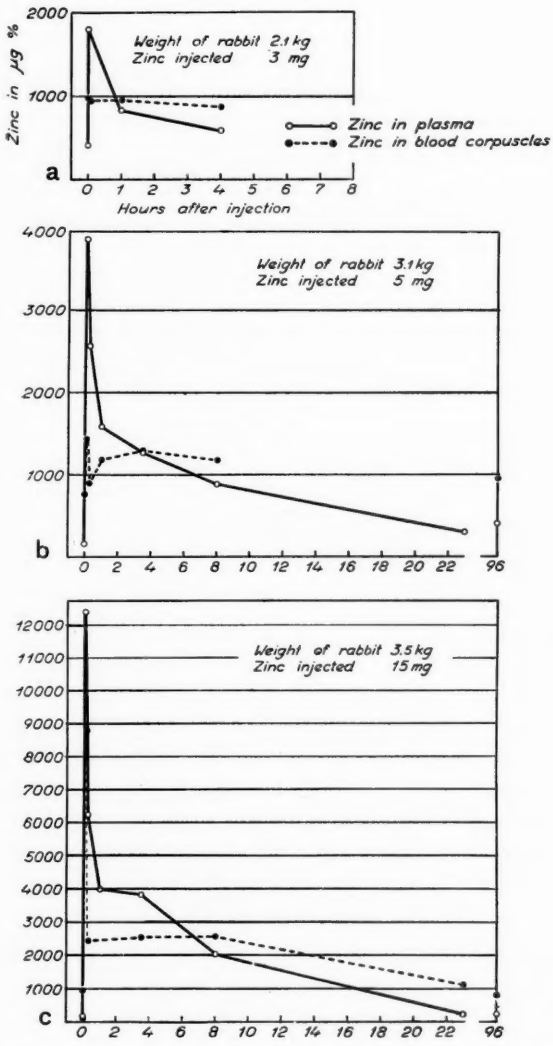

Fig. 10. *The zinc content of plasma and corpuscles in rabbits after injection of zinc as zinc acetate.*

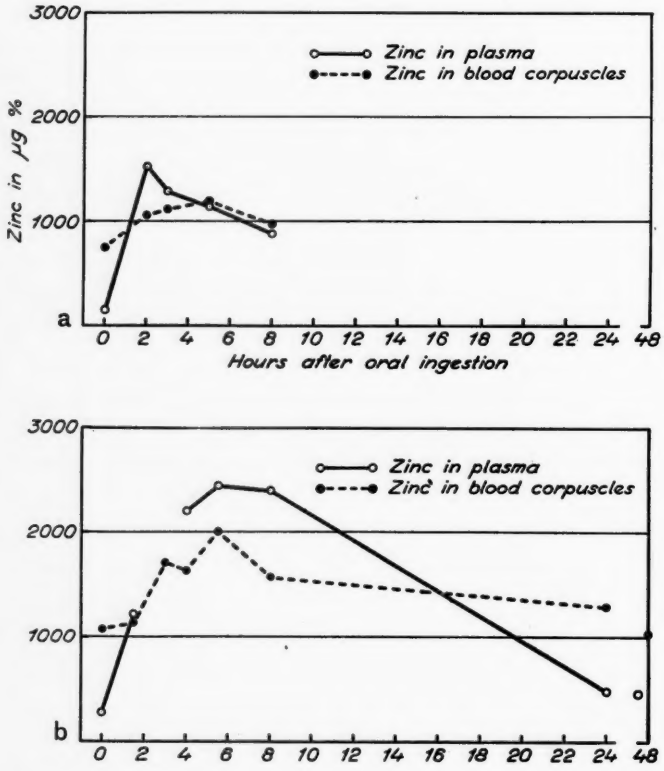

Fig. 11. *The zinc content of plasma and blood corpuscles in rabbits after oral ingestion of zinc as zinc acetate.*

In a) the dose was 400 mg. The animal was not healthy and died the following day. In b) the dose was 500 mg. The sample taken at 3 hours was a failure. The curve is thus not complete.

than that in the plasma curve, and also reaches its maximum later. It is only a temporary rise, however, and usually returns to normal within 24 hours. Nor can it be rendered more permanent by keeping the plasma zinc at a high level for a long period. When the plasma zinc values return to normal, the rise in the corpuscle values also disappears. Table XXI shows the results of

an investigation of two rabbits kept for a considerable time on a daily dose of zinc. The determinations were made after the zinc had been discontinued for at least 4 days. The plasma zinc had then returned to normal, and the same proved true of the corpuscle zinc. Samples taken 22.4. indicate determinations made while zinc diet was in progress — that is to say, when we had to allow for a temporary rise in the corpuscle zinc due to the greatly enhanced plasma zinc.

Discussion

A possible fear was that the rise in corpuscle zinc found in these tests might be due to some "contamination" from the zinc-enriched plasma; corpuscles from samples containing such zinc-rich plasma were washed at least three times with physiological NaCl solution. To prove whether this number of washings was sufficient, several blood samples from heavily zinc-enriched plasma were washed from 2 to 6 times. The samples were then analysed, and all of them yielded the same results.

Another indication that the rise in corpuscle zinc is not due either to faulty washing or to any other form of "contamination" is the difference in the behaviour of the two curves. The corpuscle zinc curve reaches its peak later than that of the plasma zinc, and subsequently maintains its level even when the zinc content in plasma is lower then that of the corpuscles, whereas, if the raised blood zinc value is really to be attributed to contamination, its peak should *coincide* with that of the plasma zinc.

Another question could also be raised: can the rise in corpuscular zinc be a consequence of a rise only in the zinc content of leucocytes? As previously mentioned the zinc determinations in corpuscles represent the values for all the cellular elements in the blood, thus also including the white blood corpuscles. VALLEE (1949) has found that the zinc content of the leucocytes is high. Thus in man, where the zinc content of the leucocytes is 25 times higher than that of the erythrocytes, 3 % of the corpuscle zinc may be ascribed to the white cells alone. In rabbits, where the zinc in the leucocyte is said to be up to 100 times more than in the erythrocyte, the white cells probably contain 10 % of the

total corpuscle zinc. VALLEE also states that in dogs a rise in the leucocyte count takes place after intravenous injection of zinc, and that the leucocytes play an important part in the zinc metabolism of the body. In spite of this it does not seem likely, however, that the effect on the corpuscle zinc seen in the present investigation is mainly due to the leucocytes. The values obtained in some of the experiments, with raised levels of the corpuscle zinc up to double the original, could hardly be explained by increase in the zinc content, or number, of the leucocytes, or both. The author has also found in a few experiments, that no leucocytosis can be found in rabbits during the first 8 hours following intravenous administration of zinc.

As regards the binding of the zinc in the blood, it has already been shown (Chapter III) that the corpuscle protein is clearly able to do this *in vitro*, the tests revealing that haemolysed corpuscles in a zinc salt solution at a neutral pH bind large quantities of the metal. On the other hand, corpuscles can evidently be kept for a long time in a heavily zinc-enriched plasma without altering their zinc content. Judged only on this evidence, it is rather surprising to find the present cases showing increased corpuscle zinc after zinc administration — the zinc in the plasma was here bound to the protein and would thus hardly be able to effect the corpuscle zinc. A possible explanation is that the corpuscles were able to absorb the zinc gradually freed from the plasma. One support for this view is the time lag between the plasma and corpuscle zinc curves mentioned above.

At the present stage of the investigation it is obviously not possible to determine definitely whether the absorbed zinc really is bound to the corpuscle protein or, which is also conceivable, whether it has been fixed in such a way that it could not be removed by washing.

In both diagrams showing the curve of the corpuscle zinc after *intravenous* administration, a large initial rise can be seen, which quickly passes off. In both diagrams the increase is apparent 3 minutes after the injection, but has already passed off in the next sample. The author has not been able to find any explanation of this phenomenon. It must be taken into account that

immediately after the injection there is a certain amount of ionized zinc present in the plasma. It is possible then that zinc can be temporarily attached to the blood corpuscles, before it is bound to the plasma proteins.

The increase of the corpuscle zinc seems to be directly dependent on the plasma zinc increase, and the present tests have yielded no upper limit for the concentrations which the former can achieve. Nor has it been possible to establish the minimum plasma zinc concentration necessary to take effect on the corpuscle zinc.

During the *long-term tolerance tests* made here, the corpuscle zinc sometimes rose to many times its original value. On these occasions it was also possible to observe macroscopic changes in the sample, the corpuscles showing an increased tendency to agglutination.

The rise in the zinc content of the corpuscles in the long-term test seems to be of temporary nature, however, since the extra zinc is rapidly eliminated as soon as the high plasma value disappears. *There consequently seems to be no way of achieving a permanent rise in the zinc content of the corpuscles.*

In five tolerance tests determinations of the enzyme carbonic anhydrase were made parallel with the zinc determinations. In view of its temporary nature, it was not surprising, that *the rise in the zinc content of the corpuscles caused no change in the activity of carbonic anhydrase.*

D. Zinc Content in the Blood of Young Rabbits after Zinc Dosage of the Pregnant and the Suckling Doe

The experiments described above indicated that it was impossible to produce a permanent rise in the corpuscle zinc of adult subjects, and it therefore remained to be seen whether, during the neonatal period, conditions were suitable for influencing the corpuscle zinc. Rabbits were again chosen, since a study of the zinc content in the plasma and corpuscles of pregnant rabbits at delivery, and of the litters (this Chapter, A) had revealed the same conditions as in man — *i. e.* subnormal plasma zinc in the

mother at delivery, with the offspring showing higher plasma zinc and lower corpuscle zinc than normal during the neonatal period. Moreover, the structure of the rabbit's placenta is known to resemble the human one, being of haemochorial type. All in all, rabbits seemed very suitable animals for the following investigations into the blood zinc of the young of zinc-fed mothers.

a. Zinc content in the blood of the young after a single intravenous dose to the dam

Our first concern was to assess the zinc permeability of the placenta by observing the effect of a single big rise in the doe's plasma zinc on that of the young. A rise of this kind is best induced by intravenous dosage, and its effect on the litter best studied immediately at birth, before the young have had time to suck.

Methods

Pregnant does were used for the tests, and the offspring was removed by caesarean section, if possible the day before parturition was expected. Anaesthesia was induced with 'Pentymal,' and maintained with ether. Blood for determining the original value of the plasma zinc was obtained by cardiac puncture. Two of the young were removed by caesarean section, and samples of their blood also taken by cardiac puncture. The doe was then given a dose of zinc acetate solution intravenously, and the injected ear was bandaged. To lessen the risk of contamination by zinc solution, the injection was given by an assistant who took no other part in the operation.

The zinc content of the doe's blood was checked from time to time. To follow possible changes in plasma and corpuscles on the foetal side of the placenta, the young were removed in pairs at intervals of $^1/_2$—1 hour, and their blood analysed for zinc. If the anaesthesia is kept at suitable depth, and temperature carefully controlled by means of a heater, the condition of the mother and also of the young, removed 6 hours after the beginning of the operation, was good.

Results

About 15 mg zinc were injected in the form of zinc acetate solution. Figure 12 shows the curves of the plasma zinc on each side of the placenta after the intravenous zinc injection. The different members in any one litter had, from the start, practically the same initial values, as is shown below. As they were removed at different intervals, they may be thought to indicate the concentration in the plasma on the foetal side of the placenta at the moment in question. It is seen, then, that *a rise in the maternal plasma* induced by relatively high intravenous zinc dosage of the mother *also causes a rise in the plasma of the young*, though of considerably smaller extent; the highest rise yet observed is one from 380 μg % to 770 μg %, corresponding to 100 %. On the other hand *no change in the corpuscle zinc* could be observed in these experiments.

The figures of the plasma zinc values obtained in the experiments have not been treated statistically in detail. When judging the result the variation between offspring of the same litter should be borne in mind. It can be seen from the Table XIX that the variation between individuals (*i.e.* without regard for consanguinity) is about 70 μg %. In the newborn rabbits this is thus about 20 % of the mean. The variation between young rabbits of the same litter is however considerably less. Investigation of 5 rabbit litters, each consisting of 2—4, showed that the standard deviation within the litters was 33 μg %. In the experiment the plasma zinc has been worked out by means of analysis of samples obtained by mixing blood from two rabbits from the same litter. Thus the standard deviation within the litter may be about 30 μg %. Consequently an increase of at least 150 μg % in the plasma zinc is statistically significant. The maximum increase in the plasma zinc in the young rabbits has in these experiments reached about 400 μg % and it is thus possible to affirm that, in all experiments, the plasma zinc rose significantly.

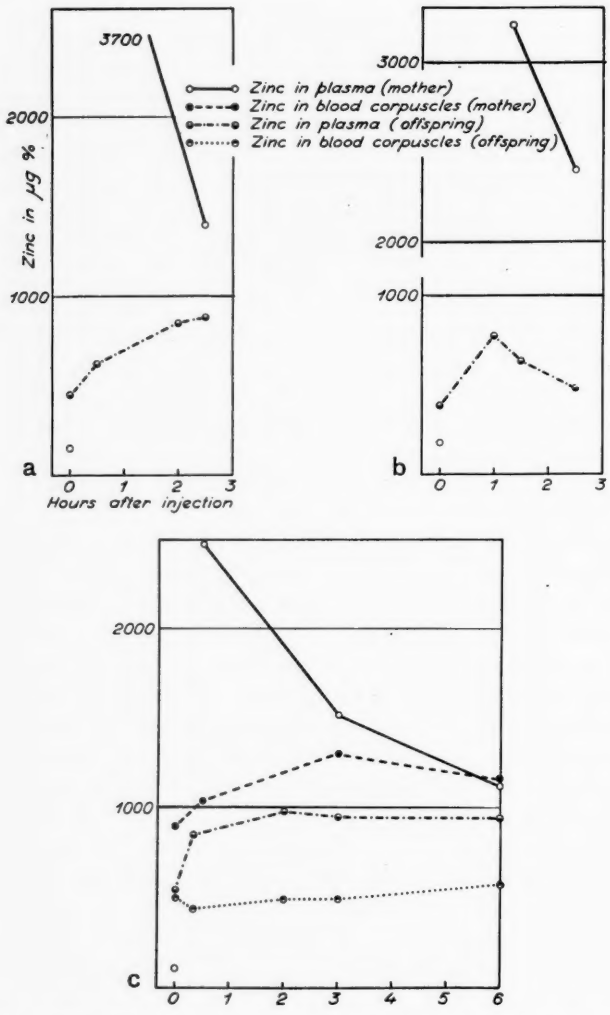

Fig. 12. *Caesarean section in rabbits. The zinc content of the blood of dam and offspring after injection of 15 mg zinc in the dam.*

b. Zinc content in the litter's blood after repeated peroral zinc dosage of the doe during pregnancy

In order to be able to influence the corpuscle zinc in the foetus it is presumably essential to maintain the plasma zinc at raised levels during the period of gestation. Previous investigations have shown that a pronounced rise in the mother's plasma zinc is accompanied by a smaller rise in that of the foetus. Thus, if the doe's plasma zinc could be kept up during the gestation period, it would mean a continuous rise in that of the foetus, also. A few attempts to give series of injections to pregnant does made the animals restless, however, so that they either distroyed their young as soon as born, or had them too early. Nevertheless, it was found that an uninterrupted rise in the plasma zinc could be obtained by repeated peroral dosage (see B page 69) and that this form of administration was both easier and less upsetting for the test animals, so that zinc was later given exclusively by mouth.

Methods

Throughout pregnancy, the does were given a daily dose of 250 mg zinc in the form of zinc acetate, in the manner already described. As soon as possible after birth, samples for zinc determination were obtained from some of the litter by cardiac puncture. Similar analyses were made from a control material, where the doe had been on normal diet, *i.e.* no additional zinc.

Results

The offspring of the dams which had received zinc showed at birth substantially higher *plasma zinc levels* (667 ± 43 µg %) than the newborn of the normal rabbits (389 ± 13 µg %). The difference is 278 ± 45 µg % and is thus statistically significant. On the other hand the *corpuscle zinc* was not influenced to any extent. The mean (587 ± 44 µg %) was hardly different from the mean in the offspring of the controls (528 ± 27 µg %). The distribution of the corpuscle zinc values among the offspring of zinc-fed and control dams is interpreted in the diagram (Fig. 13) from which it can also be seen that the increase in the corpuscle zinc during the first month is comparable in the offspring of both groups.

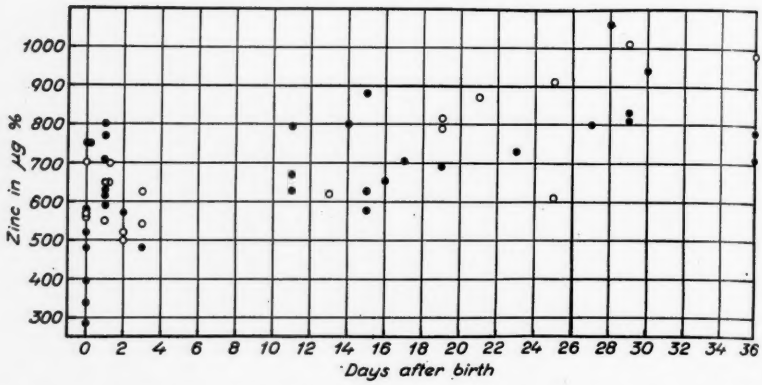

Fig. 13. *The zinc content of blood corpuscles in young rabbits at term, and at different stages during the first 36 days of life.*

Each black spot represents the corpuscle zinc in one baby rabbit, whose dam received zinc during pregnancy and lactation. The circles represent the corresponding values in the offspring of the control animals.

Discussion

As the tolerance tests on *adult animals* showed that no permanent rise in blood corpuscle zinc could be obtained from extra zinc dosage, it therefore remained to be seen whether this could be achieved by tests on *foetuses* and *newborn animals*. Prospects might well seem greater here, since there is normally a strong rise in the corpuscle zinc during gestation and the neonatal period, this rise (see above) being accompanied by an increase in the zinc-containing enzyme carbonic anhydrase.

It was a great help in this experiment that it really was technically possible to make the caesarean section in the manner described. Birth could, of course, also have been induced in the natural way, but this would have removed the opportunities of following the various phenomena, *e.g.* the course taken by the blood zinc on the foetal side over a longer period.

The rabbits were usually X-rayed before the operation to ensure that the litter was sufficiently large for a complete test. As mentioned above (page 80) the operation was performed

a day or so before delivery was expected, to lessen the chances of spontaneous birth of any of the full-term young while it was going on. The vigour of the young throughout the operation — in some cases up to 6 hours after its commencement — indicated that the circulation in the placenta must have been intact.

The tests with zinc administration during caesarean section all gave the same results. There was a relatively strong rise in the plasma zinc of the young, which in some animals was almost doubled. A certain fall was usually noticeable towards the end of the experiment, though it was moderate compared with the corresponding figure for the doe.

In a test of this kind it is, of course, always necessary to guard against contamination from the mother's zinc-laden blood, and great care was taken to see that the foetuses, removed in intact membranes, were stripped of them in a way to prevent any contact with them. The results would also be affected if there were defects in the placental barrier, either natural or caused by injury during the operation; but this was certainly not so in the present investigation, as otherwise the plasma values of the litter would definitely have shown considerably greater variations.

Our results may also give some indication of the source of the foetal zinc — namely the plasma of the doe: a logical conclusion in view of the appreciable rise in the plasma zinc of the litter demonstrable less than half-an hour after she had been injected (*cf.* Chapter VI).

Since the plasma zinc of the newborn rabbit is higher than that of the doe — a fact best shown by comparison of the two at the beginning of the caesarean section — it is plain that the passage of zinc through the placenta cannot, any more than in man, be attributed to simple dialysis.

It can be pointed out in this connection that in rabbits, as in man, the plasma zinc falls during pregnancy. This is shown by the fact that the plasma zinc in the dams just before caesarean section (179 ± 16 µg %) is lower than the normal plasma zinc (248 ± 10 µg %), the difference being 69 ± 19 µg %.

The only reported investigation of passage of zinc across the placental barrier is that of VALLEE (1949) where Zn^{65} was injected

intravenously into a pregnant bitch, after which zinc isotopes were found in the puppies. This experiment shows *that* zinc passes from mother to foetus; it does not indicate *how* the metal is transported through the placenta, or in what ways this transport might be regulated.

Another investigation of greater bearing on the metal transport through the placenta is that made by RENAER (1942) who worked with iron. He found that intravenous injection of iron into pregnant women during the last few days before delivery sent up the serum iron value to a level considerably above normal, the serum iron value of the infants, however, was not influenced.

Zinc and iron, then, seem to differ in their ability to pass through placenta to the foetal plasma. This might be explained by the different ways and concentrations in which these two metals can be bound in the plasma. Thus, the increase in the serum iron value is subject in the mother to a low saturation limit, whereas zinc can be bound in many times greater quantities.

A single raising of the mother's plasma zinc has consequently been proved to give a rise in that of the young. Now, if the mother's plasma zinc is kept at a high level throughout pregnancy, it is reasonable to suppose that that of the foetuses follows suit, and it was therefore not surprising to find that the just-born young of such does did show raised plasma zinc. Allowances must, however, be made for possible variations in the placenta's permeability to zinc at different stages of pregnancy. Such variations were shown, for example, by FLEXNER *et al.* (1948) in the case of sodium.

The tests described here have shown, then, that the conditions presumed necessary to raise the corpuscle zinc of the young have been fulfilled: the plasma zinc was shown to have increased after a single intravenous zinc injection to the mother, and in addition it seemed highly likely that the increased plasma zinc of the newborn young of the zinc-fed does indicated a continuous raised level during the foetal stage. As is shown by the results, however, it was impossible to reveal any permanent change in the *corpuscle* zinc content of the newborn animals. In the caesarean section experiments, no increase at all was noticed in the corpuscle zinc. In spite of the fact that the increase in the plasma

zinc of the mother was considerable, it was presumably not big enough to produce such a rise in the plasma zinc of the young capable to produce a rise in the corpuscle zinc. Corpuscle values from litters of zinc-fed and control does respectively did not differ, except in the case of a few slightly higher figures among the young of zinc-fed does during the first 24 hours of life — doubtless the *temporary* consequence of a high plasma zinc at or immediately before delivery.

It was earlier pointed out, that there is no possibility to cause a permanent rise in the corpuscle zinc in the *adult animal.* Despite the fact, that in *foetuses* we deal with subjects with increasing zinc content in the corpuscles, *we are even here, however, unable to do anything to accelerate this physiological increase.*

c. Zinc content in the blood of the litter during zinc dosage of the suckling dam

Zinc dosage of the pregnant rabbit has thus been shown not to affect the corpuscle zinc of the litter. It seemed worth while to continue the investigation, however, and dosage was continued during lactation, in order to follow reactions in the blood of the litter. The assumption was that the high zinc content in the doe's plasma would raise the zinc content of the milk. This would then transmit extra zinc to the young, presumably the only way of zinc administration in this case.

The blood zinc in the young of some of the litters of zinc-fed does was followed during the first month of life, during which the mother continued to receive zinc by mouth. For comparison blood zinc determinations were also made on young of does not given zinc. Samples were taken by cardiac puncture, one or — if necessary to make up the amount — two young rabbits from each litter being taken for each test.

Results

The *plasma zinc* in the offspring of dams which had received zinc was investigated during the first weeks of life (3 days—5 weeks). The series of 19 cases gave a mean of 358 ± 16 μg%. In

a series of 13 offspring of control dams, followed over the same age period, the mean plasma zinc was 247 ± 19 µg% (comparable with the values in adult rabbits, see Table XIX). The difference between the means of the two series is 111 ± 20 µg%, and is of statistical significance in spite of the small series.

It has not been possible to work out the statistics of the *corpuscle zinc* measurements made in connection with the above plasma zinc investigations because the samples were not taken at fixed intervals, and because during this time a constant increase in the corpuscle zinc is taking place. The results may be indicated by the values given in the diagram (Fig. 13), which suggest that there is no great disparity between the increases in the corpuscle zinc in the offspring of the two series.

Discussion

The zinc content in the blood of the young was followed to about the age of 5 weeks, *i.e.* during the lactation period.

The mean for the zinc content in the plasma was obtained from determinations on young rabbits aged from 3 days to 5 weeks, since the possible pre-partus influence of the doe's high plasma zinc should have been levelled out after 2 days. The higher values of the young of zinc-fed does as compared to the controls can definitely not be attributed to contamination by zinc from the mother, as otherwise the plasma values would have shown a totally irregular dispersion.

The higher plasma zinc mean of the first group would indicate that they were supplied with extra zinc through the *milk*. A rise in the zinc content of the milk was expected, since other animals were known to have shown a similar reaction after zinc dosage. LEVALD (1857) and ARCHIBALD (1943), for example, have shown a considerable increase of this metal in the milk of cows to whose food it had been added in large quantities. The present author, however, was not able to find any wholly satisfactory method of drawing off rabbit milk for zinc analysis.

This rise of the milk's zinc content which can thus be assumed to follow zinc dosage of the mother is probably linked up with the ability of the plasma to bind large quantities of this metal — a

thing it is not able to do in the case of iron and copper (Chap. III), as shown, for example, by experiments with peroral dosage of iron to nursing mothers (WALLGREN 1931) or intravenous injections of copper (MUNCH-PETERSEN 1950). In neither case did the milk show any increase of the metal in question. Nor was any enrichment of the milk found, in animal experiments, after feeding these metals to the does (ELVEHJEM *et al.* 1927 and 1929, HENRIQUES & ROCHE 1929).

It has previously been stated that some of the young of does fed on zinc during pregnancy showed somewhat higher corpuscle values than the controls at the actual moment of birth. As the diagram shows, however, the difference was soon levelled out, and the subsequent rise in the corpuscle values ran parallel in both groups, though the young in the first group (*i.e.* with zinc-fed mothers) must have been getting more of the metal, through the milk, than the others. Though the material is small, the result seemed conclusive enough to allow us to dispense with further time-consuming experiments of the same kind. It appears, then, that *the gently rising zinc content in the blood corpuscles during the first period of life is impossible to influence through addition of extra zinc to the food.*

Summary

1. The author has introduced a modification in *the methods* usually adopted in the past for zinc analysis. Instead of ashing the material the zinc is extracted with strong hydrochloric acid and the protein precipitated with trichloroacetic acid. Zinc determination is performed on the clear supernatant fluid using a dithizone method.

2. The *stability of the zinc protein combination* at different degrees of acidity has been studied in dialysis tests. The zinc in both plasma and haemolysed corpuscles separated in increasing amount from the protein complex when the pH value fell below the neutral point.

3. The *capacity* of the plasma and haemolysed blood corpuscles *to bind additional zinc* was studied in dialysis tests. It proved to be very high indeed admitting non-dialysable combination even in concentrations of zinc very many times higher than normal. Thus, contrary to iron, the plasma does not show a saturation limit for zinc within the range of this investigation.

4. The *plasma zinc* in 126 healthy adults shows an average level of 109 ± 2 µg %.

The plasma zinc level in 10 foetuses between the 4th and 6th months is 300 ± 31 µg %, in 16 premature infants 187 ± 20 µg %, in 39 full-term newborn infants 125 ± 5 µg %. Later in infancy the plasma zinc level is somewhat higher than the adult value. Thus the plasma zinc shows a continuous fall from the very high values during the foetal stage.

During pregnancy the plasma zinc shows a definite fall with values 20—25 % below normal at the end of gestation.

5. The *zinc content of the blood corpuscles* in 84 healthy adults was 1244 ± 20 µg %.

During the foetal stage the zinc content of the blood corpuscles is very low and rises only slowly, with a mean value at term of 376 ± 19 µg %. After birth the values initially rise more rapidly, values of 800 µg % being reached by the end of the first year, and adult level by adolescence.

6. A comparison between *mother and child at birth* shows higher plasma values but much lower blood corpuscle values in the child as compared with the mother.

The possible transfer of zinc from mother to foetus by means of the plasma zinc is discussed.

7. There is a correlation between the behaviour of blood *corpuscle zinc* and that of *carbonic anhydrase*, both showing a similar succesive increase with age.

8. The infant receives considerable quantities of *zinc from the food*. Analyses of breast milk revealed that the zinc concentration varied. In colostrum it is as high as 2000 µg %, after a month or two 300—500 µg %, and later on it falls still lower.

Balance tests performed on a limited number of children indicated that the organism does not retain more than small quantities of the zinc supplied with the food.

9. *In experiments on rabbits* the following observations were made.

a) Intravenous zinc dosage in the form of zinc salt solution gives a considerable increase in the zinc of the circulating blood. Rabbits of normal size tolerate a dose of 20—30 mg zinc, which quantity gives plasma zinc values of over 10 000 µg %.

b) Oral administration of zinc salt gives a definite but transient rise in the plasma zinc. After prolonged oral adminstration of zinc a single dose of zinc produces higher plasma values that persist for a considerable time. 0.25 g zinc daily is tolerated by adult animals for a long period without any toxic signs.

c) Intravenous as well as oral zinc administration gives a rise not only in the plasma zinc level but also in the content of zinc in the blood corpuscles. The high corpuscle zinc persists only as long as the plasma zinc values are definitely raised. The increase of the zinc content of the blood corpuscles thus provoked is not reflected in an increased carbonic anhydrase activity.

d) In experiments with pregnant rabbits it was demonstrated that an increased plasma zinc level in the dam gives rise to an increased plasma zinc level in the offspring. This increased level in the plasma does not lead to *persistently* high levels in the corpuscle zinc of the newborn animals.

e) The progressive increase in corpuscle zinc of the young animals during the first month of life does not seem to be affected by artificially increased zinc intake with the food.

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Zusammenfassung.

Studien über das Blutzink.

Eine experimentelle und klinische Untersuchung über den Zinkgehalt des Plasmas und der Blutkörperchen mit besonderer Hinsicht auf die Verhältnisse bei Kindern.

1. Der Verfasser hat eine Modifizierung der früher gebräuchlichen *Methoden* für Zinkanalysen ausgearbeitet. Anstatt das Material einzuaschen, wird das Zink mit starker Salzsäure extrahiert und das Eiweiss mit Trichloressigsäure gefällt. Die Zinkbestimmung geschieht dann in der klaren Lösung mit einer Dithizonmethode.

2. Die *Stabilität* des Zink-Eiweiss-Komplexes bei verschiedenen Säuregraden wurde in Dialyse-Versuchen studiert. Das Zink spaltete sich von dem Eiweisskomplex in umso höherem Grade ab, je tiefer das pH unter den Neutralpunkt sinkt. Dieses Verhalten gilt sowohl für Plasma als auch für hämolysierte Blutkörperchen.

3. Weiter wurde die *Fähigkeit* des Plasmas und der hämolysierten Blutkörperchen, *zusätzliches Zink zu binden*, in Dialyse-Versuchen studiert. Diese erwies sich ausserordentlich gross und ergab nicht dialysierbare Verbindungen auch bei Zinkkonzentrationen, die den Normalwert vielfach übertrafen. So zeigt das Plasma, im Gegensatz zum Verhalten gegenüber Eisen, keine Sättigungsgrenze für Zink bei den hier untersuchten Konzentrationen.

4. *Das Plasmazink*, untersucht bei 126 gesunden Erwachsenen, liegt durchschnittlich bei $109 \pm 2\ \gamma\%$.

Die Bestimmung des Plasmazinkspiegels bei 10 Fötussen zwischen 4 und 6 Monaten ergab $300 \pm 31\ \gamma\%$, bei 16 Frühgeburten $187 \pm 20\ \gamma\%$, bei 39 ausgetragenen Neugeburten $125 \pm 5\ \gamma\%$. Während der ersten Kindheit liegt der Zinkspiegel etwas höher als bei Erwachsenen. Das Plasmazink zeigt also ein stetiges Absinken von dem hohen Werte unter der Fötalperiode.

Während der Schwangerschaft zeigt das Zink ein deutliches Absinken auf Werte, die am Ende der Schwangerschaft 20—25% unter dem Normalwerte liegen.

5. *Der Zinkgehalt der Blutkörperchen*, untersucht bei 84 gesunden Erwachsenen, liegt bei $1244 \pm 20\ \gamma\%$.

Während der Fötalperiode ist der Zinkgehalt der Blutkörperchen sehr niedrig und steigt nur langsam an, der Durchschnittswert liegt bei der Geburt bei $376 \pm 19\ \gamma\%$. Nach der Geburt steigt der Wert anfänglich etwas schneller, erreicht am Ende des ersten Lebensjahres $800\ \gamma\%$ und erst im spätesten Kindesalter den Wert der Erwachsenen.

6. Ein Vergleich zwischen *Mutter und Kind bei der Geburt* zeigt höhere Plasmazinkwerte, aber viel niedrigere Blutkörperchenzinkwerte bei dem Kinde.

Die Möglichkeit, dass das Zink des Fötusses von dem Plasmazink der Mutter herstammt, wird diskutiert.

7. Es besteht eine Übereinstimmung in dem Verhalten des *Blutkörperchenzinks* und dem der *Kohlensäureanhydratase*, indem beide mit steigendem Lebensalter ein allmähliches Ansteigen zeigen.

8. Das Kind erhält *ansehnliche Mengen Zink mit der Nahrung.* Brustmilchuntersuchungen zeigten einen absinkenden Zinkgehalt: im Kolostrum ist dieser $2000\ \gamma\%$, nach ein bis zwei Monaten $300—500\ \gamma\%$ und fällt später auf noch tiefere Werte.

Bilanzversuche, ausgeführt bei einer kleinen Zahl von Säuglingen, lassen vermuten, dass der Organismus nur kleine Mengen des mit der Nahrung zugeführten Zinks behält.

9. Bei *Kaninchenversuchen* wurden folgende Beobachtungen gemacht.

a. Intravenöse Zinkgaben in Form von Zinksalzlösungen führen zu erheblicher Steigerung des Zinkgehaltes im zirkulierenden Blute. Kaninchen von normaler Grösse tolerieren eine Gabe von 20—30 mg Zink, welche Menge den Plasmazinkgehalt auf über $10000\ \gamma\%$ erhöht.

b. Orale Zinksalzgaben verursachen einen deutlichen, aber vorübergehenden Anstieg des Plasmazinks.

Bei fortgesetzter oraler Zinkzufuhr gibt jede neue Zinkgabe

höhere Plasmazinkwerte, die dann auch längere Zeit bestehen bleiben. 0,25 mg Zink täglich wird von erwachsenen Tieren während längerer Zeit ohne toxische Beeinträchtigung ertragen.

c. Intravenöse wie auch orale Zinkzufuhr erhöht nicht nur den Plasmazinkspiegel, sondern auch den Zinkgehalt der Blutkörperchen. Dieser wiederum bleibt erhöht nur solange der Plasmazinkgehalt deutlich gesteigert ist. Die so erzielte Zinkanreicherung in den Blutkörperchen verursacht aber nicht eine Steigerung in der Aktivität der Kohlensäureanhydratase.

d. In Versuchen mit schwangeren Kaninchen wurde gezeigt, dass ein erhöhter Plasmazinkgehalt bei den Muttertieren auch einen erhöhten Plasmazinkgehalt bei den Nachkommen verursacht. Dieser erhöhte Plasmazinkgehalt führt nicht zu bestehenden hohen Werten des Blutkörperchenzinks bei den neugeborenen Tieren.

Der normale Anstieg des Zinkgehaltes in den Blutkörperchen neugeborener Tiere während des ersten Lebensmonats scheint durch künstlich gesteigerte Zinkzufuhr mit der Nahrung nicht beeinflusst zu werden.

Résumé.

Études sur le zinc du sang.

Recherches expérimentales et cliniques sur la teneur en zinc du plasma et des globules du sang, en égard particulièrement aux conditions de l'enfance.

1. L'auteur a introduit une modification des *méthodes* employées auparavant pour l'analyse du zinc. Au lieu de calciner la matière, on extrait le zinc à l'aide d'un acide chlorhydrique fort, et l'on précipite la protéine à l'aide d'acide trichloroacétique. La détermination du zinc est exécutée ensuite sur la solution claire avec une méthode de dithizone.

2. La *stabilité du complexe zinc-protéine* a été étudiée à différents degrés d'acidité dans les expériences de dialyse. Le zinc, dans le plasma et les globules du sang, s'est séparé du complexe protéique à un degré d'autant plus élevé que le pH descend plus bas au

dessous du point neutre. Cela est valable aussi bien pour le plasma que pour les protéines des globules du sang.

3. De plus, la *capacité* du plasma et des protéines des globules du sang de *lier un supplément de zinc*, a été étudiée dans les expériences de dialyse. Cette capacité est apparue extraordinairement grande et a permis des combinaisons non dialysables même dans des concentrations de zinc qui surpassent de beaucoup la valeur normale. Ainsi le plasma ne montre, au contraire de sa réaction à l'égard du fer, aucune limite de saturation pour le zinc dans les concentrations étudiées ici.

4. Le *zinc du plasma*, étudié chez 126 adultes bien portants, se trouve en moyenne à $109 \pm 2 \mu g\,\%$. Le niveau du zinc du plasma, chez 10 foetus entre le quatrième et le sixième mois, est de 300 ± 31 $\mu g\,\%$, chez 16 enfants prématurés, de $187 \pm 20 \mu g\,\%$, chez 39 nouveaux nés arrivés à terme, de $125 \pm 5 \mu g\,\%$. Pendant la première enfance, le niveau est un peu plus haut que chez les adultes. Ainsi, le zinc du plasma montre une chute continue à partir des très hautes valeurs du stade foetal.

Pendant la gravidité, le zinc plasmatique montre une dépression marquée, avec des valeurs qui sont de 20 à 25 % au-dessous de la normale à la fin de la gestation.

5. La *teneur des globules du sang en zinc* chez 84 adultes bien portants est de $1244 \pm 20 \mu g\,\%$.

Au stade foetal, la teneur des globules en zinc est très basse et monte très lentement, avec une valeur moyenne de $376 \pm 19 \mu g\,\%$ à la naissance. Après la naissance, les valeurs montent d'abord plus rapidement, atteignant 800 $\mu g\,\%$ à la fin de la première année, et le niveau adulte est atteint à l'adolescence.

6. Une comparaison entre *la mère et l'enfant à la naissance* fait apparaître des valeurs de zinc plasmatique plus hautes, mais de zinc globulaire beaucoup plus basses chez l'enfant que chez la mère.

On discute la possibilité de la transmission du zinc de la mère au foetus par le zinc plasmatique.

7. Il y a une corrélation étroite entre l'attitude du *zinc des globules du sang et celle de l'anhydrase carbonique*, en ce que tous deux montrent une croissance progressive semblable suivant l'âge.

8. L'enfant reçoit par la *nourriture des quantités considérables de zinc.* Des analyses de lait de femme révèlent que la concentration de zinc varie: dans le colostrum, elle est de 2000 μg %, après un ou deux mois de 300—500 μg %, et plus tard elle tombe encore plus bas.

Des expériences de balance, faites sur un nombre limité d'enfants, font penser que l'organisme retient seulement de petites quantités du zinc fourni par la nourriture.

9. Dans des *expériences* réalisées sur *les lapins*, on a fait les observations suivantes:

a. Une injection intraveineuse de zinc sous forme d'un solution de sel de zinc entraîne une augmentation considérable du zinc dans le circuit sanguin. Des lapins adultes supportent une dose de 20 à 30 mg de zinc, quantité qui donne des valeurs de zinc plasmatique de plus de 10 000 μg %.

b. une administration per orale de zinc amène une montée marquée mais passagère du zinc plasmatique. Après un traitement prolongé du zinc, un dosage isolé produit des valeurs de zinc qui sont plus hautes et se maintenent plus longtemps. Des animaux adultes tolèrent 0,25 mg de zinc par jour pendant longtemps, sans aucun signe d'intoxication.

c. Une injection intraveineuse aussi bien qu'une administration per orale amènent une montée non seulement du niveau de zinc plasmatique mais aussi de la teneur des globules en zinc. Cette teneur ne persiste qu'aussi longtemps que les valeurs du zinc plasmatique sont nettement élevées. L'augmentation ainsi provoquée dans la teneur des globules sanguins en zinc ne produit aucun accroissement de l'activité d'anhydrase carbonique.

d. Dans des expériences réalisées avec des lapines pleines, il a été démontré qu'une élévation du niveau du zinc plasmatique chez la mère entraîne une même élévation chez le petit. Cette élévation ne produit pas de niveaux continuellement élevés dans le zinc des globules des animaux nouveaux-nés.

L'accroissement progressif du zinc des globules du sang chez les jeunes animaux pendant le premier mois de leur vie ne semble pas être influencé par une administration artificielle accrue de zinc avec la nourriture.

Resumen.

Estudios sobre el zinc de la sangre.

Investigaciones experimentales y clínicas sobre el contenido de zinc en el plasma y en los glóbulos de la sangre, especialmente en la infancia.

1. El autor ha introducido una modificación en *los métodos* empleados hasta la fecha para el análisis del zinc. En lugar de calcinar la materia, se extrae el zinc por medio de un ácido clorhídrico concentrado, y se precipita la proteína con ácido tricloroacético. La determinación del zinc se lleva enseguida a efecto en la solución clara por un método de dithizona.

2. *La estabilidad de la combinación zinc-proteína* ha sido estudiada, en diferentes grados de acidez, en experimentos de diálisis. El zinc en el plasma y en los glóbulos sanguíneos, se separa de la combinación proteica a un grado tanto más elevado cuanto el pH llega más por debajo del punto neutro. Esto sirve también para el plasma, igualmente que para las proteínas de los glóbulos de la sangre.

3. Además, la *capacidad* del plasma y de las proteínas de los glóbulos sanguíneos *para absorber un suplemento de zinc*, ha sido estudiada en los experimentos de diálisis. Esta capacidad se ha manifestado extraordinariamente grande, y ha permitido combinaciones no dializables, incluso en concentraciones de zinc que sobrepasan en mucho el valor normal. Así el plasma no muestra, contrariamente a su reacción con respecto al hierro, ningún límite de saturación para el zinc, en las concentraciones aquí estudiadas.

4. El *zinc del plasma*, estudiado en 126 adultos, de buena salud, se encuentra en una media de 109 ± 2 µg %. El nivel del zinc del plasma en 10 fetos, entre el cuarto y el sexto mes, es de 300 ± 31 µg %; en 16 niños prematuros, de 187 ± 20 µg %; en 39 recién nacidos llegados a término, de 125 ± 5 µg %. Durante la primera infancia, el nivel es un poco más alto que en los adultos. Así, el zinc del plasma muestra un descenso continuo a partir de las muy altas cifras del estado fetal.

Durante la gravidez, el zinc plasmático acusa un marcado descenso, con valores que bajan de 20 a 25 % por debajo de la normal, al terminar la gestación.

5. El *contenido de zinc en los globulos* de la sangre en 84 adultos sanos es de $1{,}244 \pm 20$ µg %.

En estado fetal, este contenido es muy bajo y aumenta muy lentamente, con un valor medio de 376 ± 19 µg% al nacer. Después del nacimiento, los valores suben, al principio muy rápidamente, alcanzando 800 µg % al finalizar el primer año, y el nivel adulto se alcanza en la adolescencia.

6. Una comparación entre *la madre y el hijo al nacer*, descubre más altos valores de zinc plasmático, pero mucho más bajos de zinc globular, en el niño que en la madre.

Se discute la posibilidad de la transmisión del zinc de la madre al feto por el zinc plasmático.

7. Existe una estrecha correlación entre el desarrollo del *zinc globular* y la de la *anhidrasa carbónica*: ambos crecen igualmente de un modo progresivo con los años.

8. El niño recibe cantidades considerables de *zinc por medio del alimento*. Los análisis de leche de mujer revelan que la cantidad de zinc varía: en el calostro es de 2,000 µg %, después de uno o dos meses de 300—500 µg %, y más tarde baja todavía más.

Las pruebas de asimilación llevadas a cabo con un número limitado de niños, hacen pensar que el organismo retiene sólamente pequeñas cantidades del zinc suministrado por el alimento.

9. En *experimentos* realizados *con conejos*, se ha observado lo siguiente:

a. Una inyección intravenosa de zinc, en forma de solución de sal de zinc, produce un aumento considerable del zinc en el circuito sanguíneo. Los conejos adultos soportan una dosis de 20 a 30 mg de zinc, cantidad que da valores de zinc plasmático de más de 10,000 µg %.

b. Una administración, por vía oral, de zinc, produce una subida, marcada pero pasajera, de zinc plasmático. Un tratamiento prolongado de zinc, por vía oral, produce valores de zinc plasmático más altos y estables. Los conejos adultos toleran dosis diarias de 0,25 mg de zinc, durante un largo período, sin el menor síntoma de intoxicación.

c. Una inyección intravenosa, así como una administración por vía oral, producen, no sólamente una subida del nivel del

zinc plasmático, sino también del contenido de zinc en los glóbulos sanguíneos. Dicha subida no persiste más que mientras los valores del zinc plasmático son francamente elevados. El aumento así provocado del contenido de zinc en los glóbulos sanguíneos, no tiene correspondencia con un crecimiento de la anhidrasa carbónica.

d. En los experimentos llevados a cabo con conejas grávidas, se ha demostrado que una elevación del nivel del zinc plasmático en la madre, produce una misma elevación en el pequeño. Esta elevación no produce niveles *continuamente* elevados en el zinc de los glóbulos de los animales recién nacidos.

El aumento progresivo de zinc en los glóbulos sanguíneos en los animales jóvenes, durante el primer mes de su vida, no parece estar influído por el aumento artificial de zinc proporcionado con el alimento.

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THE PREMATURE CHILD

THE INCIDENCE, ÆTIOLOGY, MORTALITY AND THE FATE OF THE SURVIVORS

BY
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PREFACE

The material was collected and the greater part of this study completed when I was assistant at the Institute of Hygiene of the University of Oslo.

During my work I have been helped by many whose names cannot be mentioned here, but I will thank them all for their kind help.

I owe especial thanks to my former chief, professor Axel Strøm, who has given me advice from beginning to end. I also thank Miss Thordis Nakstad of the University Institute of Hygiene for valuable assistance.

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Oslo, July 1952.

Sigrid Daae Blegen.

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I. INTRODUCTION

In 1919 Arvo Ylppö published his extensive monograph on premature babies. This publication was the result of detailed studies of the anatomy, physiology and clinical aspects of prematures.

He found a very high mortality during the first year of life (about 50 %). During the first month after birth mortality was about 31 %, and during the first day about 9 %.

He also observed delayed growth in all surviving premature babies as a result of injuries sustained at birth or later. In most cases this delay was compensated during the first year of life, but in children with the lowest birth weights it persisted until 5—6 years of age. Some never attained average weights. He found that 7.4 % were idiots and imbeciles, and 3.1 % had affections of the central nervous system, Little's disease etc.

His conclusion was that many of those who survived to maturity were not perfectly normal individuals.

A number of publications on premature babies appeared in Europe and America during the 1920's and 1930's and most of them were characterized by the same pessimistic outlook regarding premature babies and their developmental prospects.

Salomonsen (1926) made a control examination of 64 prematurely born children who had been hospitalized at Dept. VIII of Oslo city Hospital and came to the following conclusion: "Even if their physical condition is good, a large number of these children are psychically defective individuals." (21 % mentally retarded.)

Capper (1928) found that the prognosis for the premature babies was very poor, both mentally and physically, and poorest for the very small babies with a birth weight of 1500 grams or less (immature). He found 7 % idiots and 5 % with Little's disease. Only ⅓ of the school children were in classes corresponding to their age and 11 % were in special schools. He found further that morbidity was increased during the first year of life, and congenital defects were more frequent than among other babies.

Sunde (1930) examined 559 prematurely born up to the age of 21 years, most of them between 10 and 15 years of age. He found a somewhat lower mortality during the first year of life (35.8 %) than Ylppö, but the latter's material included a greater number of babies in the lowest weight classes, where mortality is highest. Among the children who grew up 7 % were defective, 5.7 % were mentally defective. A supplementary investigation made by C. Schiøtz and J. Lofthus showed that the children were below average in both physical and mental development.

Brander (1935—41) came to similar results. In a follow-up examination of 376 premature babies he found that they were less than others in height and weight, and that their mental development was inferior to the average for children of the same age. He found no tendency to compensation toward the end of school age. He found that 11.2 % of the children were mentally defective (I. Q. $\leq$ 70).

An investigation by Hess (1934) is an exception to other publications of this period. When he used the sibs of the premature babies as control he found no difference in height, weight or mental development.

While the publications from the 1920's and 1930's take a pessimistic view of the developmental possibilities of the premature babies, a number of investigations after 1940 give a somewhat different impression. In principle they follow the same direction as Hess' study, and emphasize particularly the significant rôle of environmental conditions for the development of the premature babies. It is pointed out that the same unfavorable social and economic conditions

under which the mothers live during pregnancy and which contribute to premature birth, also affect the child after birth and retard its development. It is thus not only the premature birth, but just as much poor social conditions which are responsible for the retarded development of these children.

Only a few of the publications in which this viewpoint is presented will be mentioned:

Levine and Gordon (1942) found no significant difference between premature and full term children as regards height and weight. These prematurely born had grown up under good social conditions.

Cecil Mary Drillien (1948) followed up premature babies in Edinburgh to school age and found a difference in development corresponding to the various weight groups, poorest for those with the lowest birth weight. Social conditions played a significant rôle for the development of the children.

Hedwig Koenig (1950) made follow-up examinations of 700 premature children in New York who had been controlled at a special health clinic for premature babies. She found in regard to teething, walking, talking, toilet training etc. that these children were just like average children. Only 2 % were mentally defective. She found that when the babies receive the proper care, and when the mothers are instructed and have necessary help, the results are considerably better than they were previously. Even the smallest babies will have a chance to attain normal height and weight, though they *may* be somewhat delayed in their development.

Other investigations along the same trend are those of Brown, Lyon and Anderson (1946).

Thus there has been a marked change in the interpretation of the developmental prospects of premature babies. The results of follow-up examinations of premature babies who have received proper care since birth and who have reported for control at special clinics for prematurely born, provide a basis for a much more optimistic view of their prospects than was previously possible. The problems in connection with premature babies have thus changed in aspect.

No investigation of premature babies has been made in Norway during the last 20 years. During this period social conditions have changed considerably, and knowledge concerning infant and maternal care has become far more widespread. However there has been no opportunity of providing special treatment for premature babies similar to that which is practised in some places in America.

The author believes that it would be of interest to determine whether these factors have led to any changes in the developmental possibilities of premature babies in this country similar to those in other countries. The aim of this investigation is to determine the fate of the premature babies born in Oslo from 1930—1939, some of whom are now adults. The mothers of these children had the opportunity of seeking advice and direction at clinics for maternal and infant care, and a general knowledge of maternal and infant hygiene may be assumed during the period in which these children were born.

The questions taken up for investigation are:

The frequency of premature births in Oslo from 1930—1939.
Causes. (Multiple births, mother's age at birth, the child's birth number, mother's health during pregnancy, and social conditions.)
Mortality among the premature. (Stillbirths, neo-natal and subsequent mortality.)
Development of the survivors. (Physical and mental.)
Influence of social conditions on this development.

II. DEFINITION OF PREMATURE BIRTH

Ylppö used birth weight as a basis for his definition of a premature baby. He found that it was difficult to measure the length of a newborn infant with accuracy, and that information on the duration of pregnancy as obtained from the mother was unreliable.

He defined a premature baby as one with a birth weight of 2500 grams or less. This definition has been accepted in

recent years to an increasing extent. It was recognised in 1935 by The American Academy of Pediatrics, in 1937 by an international committee in Geneva, and in 1938 by The Royal College of Physicians of London and The Royal College of Gynæcologists and Obstetricians of England.

Obviously, among infants with a birth weight of 2500 grams there will be a few who are full term, but in general the definition will be in accord with the clinical findings.

The definition is more doubtful with regard to twins, but is also generally applied here.

The lower limit toward miscarriage still varies from country to country, but efforts toward greater uniformity are being made. The most common practice has been to classify any delivery where the pregnancy has been of shorter duration than 28 weeks as a miscarriage. This corresponds to a birth weight of about 1250 gm and a length of about 35 cm. This limit is still used in several countries, e. g. in Great Britain and Norway.

In recent years it has been found that many infants with a low birth weight survive with the help of the modern special care available for premature infants. It has thus been necessary to revise the conception of miscarriage.

At The Scandinavian Pediatric Congress in Helsinki in 1946, Ylppö suggested the following classification:

Premature: 2500—1250 gm.

Immature: 1250—600 gm.

Miscarriage: Less than 600 gm.

This classification is now used in Finland.

The designation immature or previable for the smallest prematures has also been employed previously, with a rather variable upper weight limit, usually 1500 gm.

Since 1930 in the United States the lower limit toward miscarriage has been 20 weeks according to a regulation for The Bureau of Vital Statistics, but since 1943 the limit has been lowered to 16 weeks: 400 gm.

In the present publication all babies with a birth weight between 2500—400 gm are considered premature.

III. COLLECTION OF DATA

The data on premature babies born in Oslo during 1930—1939 to mothers who lived in the city were gathered from the following sources:

1. Oslo Bureau of Public Health's birth reports. All birth reports for the years 1930—1939 were examined and data recorded for women residing in Oslo.

2. Case records from the public maternity clinics in Oslo (The University Maternity Clinic, Oslo Municipal Maternity Clinic, Kroghstøtten Maternity Dept.) for this period.

The case records contain information on the health of the mother during pregnancy, her marital status, address before delivery, where she goes when she leaves the clinic and whether she is a resident of Oslo. There is also information as to the number of previous births (previous miscarriages), the course of the delivery, *birth weight* (usually also recorded when the birth is considered a miscarriage according to Norwegian regulations), the child's condition at birth and development while at the clinic, and any congenital defects are recorded. If the child is still-born or dies subsequently at the clinic, the cause of death is recorded when possible together with possible autopsy findings.

These data were recorded for all babies with a birth weight of between 2500 and 400 gm born to mothers residing in Oslo — a total of 1127.

3. Oslo Register of Persons. Information was obtained as to which children had died after discharge from the clinic. For these children as well as those which had died at the clinic, the *death reports at the Bureau of Public Health* were examined, and cause of death, age at death etc. were recorded.

The address of all children alive on January 1, 1948 was obtained through the Register of Persons. There were 682 such children and 541 of them (79.3 %) were found. No information could be obtained on 141 children because some

had been adopted immediately after discharge from the clinic, others had moved out of town, and in a few cases the mother refused to give further information (see below).

4. *Direct questioning.* Visits were made to the homes of those children who were alive in 1948 and who had been traced through the Register of Persons. From parents or other relatives, or sometimes directly from the child, information was obtained as to:

A. The child's development from birth and later, nourishment, control at infant clinic or regular visits to doctor, schooling, etc.

B. Diseases suffered and any accidents (possible physical anomalies).

C. Social conditions under which the child grew up, especially housing conditions. Most of them were willing to give information as to the family's financial situation, e. g. if they had been supported by public charity.

D. Particular diseases in the family, especially mental diseases, epilepsy, alcoholism.

In this manner information on 525 of 541 children was obtained. The information obtained from the mothers was supplemented by examination of:

5. The school health cards which contain information on the health of the children during school years, results of the annual weighing and measuring, and whether the child has been examined by any specialist, among them the school psychiatrist.

6. *The child's school record etc.* For those children who had attended regular schools, information was obtained from teachers as to their scholastic ability, and the teachers' opinion of the children's character. For this information a form was used which is filled out by teachers since 1947 for the purpose of occupational guidance for all children who leave the primary schools. The form is printed as an appendix to the present publication (page 68). Such information was collected for 476 premature children.

7. *Information from institutions such as homes for mental defectives, corrective schools, special schools, homes for crippled children.* For those children who had been or still were at such institutions, information was obtained from the institution's physician or head. For pupils at special schools, information was obtained from the health cards where the result of the psychiatric examination is always attached.

IV. THE FREQUENCY OF PREMATURE BIRTH IN OSLO DURING THE PERIOD 1930—1939, BASED ON MUNICIPAL BIRTH RECORDS

The birth reports to the Bureau of Public Health include live born babies and those which are called "born dead" according to the Norwegian system, i. e. those which are stillborn and those who die within 24 hours. Fætuses under 28 weeks are not reported.

During 1930—39 a total of 23 618 children were born in Oslo to mothers resident in the city. 22 771 children were born alive and 847 "born dead". Table 1 shows the distribution of these children in public and private clinics and born at home.

It appears that more than 70 % of the children born in Oslo to mothers resident in the city during the period 1930

Table 1. *Distribution of births in Oslo 1930—39 at public and private maternity clinics and at home.*

Children born in Oslo 1930—39	Born alive		Born »dead«		Total		»Born dead«
	n	%	n	%	n	%	% of total
Public clinics	16376	72.0	555	65.5	16931	71.6	3.3
Private clinics ...	2603	11.4	86	10.2	2689	11.4	3.2
At home	3792	16.6	206	24.3	3998	16.9	5.2
Total	22771	100.0	847	100.0	23618	99.9	3,6

Table 2. *Frequency of births in 5th—8th month of pregnancy in Oslo 1930—39.*

Children born in Oslo 1930—39	Total	Born alive in 5th—8th month		Total 'born dead'	»Born dead« in 5th—8th month		Total born in 5th—8th month	
		n	%		n	%	n	%
Public clinics	16376	452	3.1	555	213	38.3	665	4.0
Private clinics	2603	73	2.5	86	27	30.4	100	3.7
At home ...	3792	121	3.2	206	122	50.8	243	6.1
Total	22771	646	2.8	847	362	47.1	1008	4.6

—1939 were born at public clinics. Only about 17 % were born at home.

On the birth reports for the live born children, the birth weight is not recorded, but the duration of pregnancy is. For those "born dead" the birth weight is also recorded. According to the reports, children born during the 5th to 8th month are classified as premature.

Table 2 shows the frequency of births of this type.

This shows that premature births are somewhat more frequent at home than at the clinics. Private and public clinics give about the same figures.

The difference is due largely to a greater number of prematures among the "born dead" which were delivered at home as compared to the clinics. For those born alive the situation is practically the same for all 3 groups. As the live born comprise about 96 % of all births, it should be justifiable to regard the children born at public clinics as representative for all children born in Oslo during the period covered by the investigation. In the following, therefore, the investigation is concentrated mainly on these children.

V. THE FREQUENCY OF PREMATURE BIRTHS AT PUBLIC CLINICS IN OSLO 1930—1939

As mentioned in the preceding chapter, children born at public clinics comprise 72 % of all children born in Oslo during the period 1930—39. Examination of the case records at the clinics revealed some contradictions between the information contained here and the birth reports. Thus in the records at the clinics there were 76 "born dead" for which no reports had been sent in. Most of these had a birth weight under 1000 gm so pregnancy had been of shorter duration than 28 weeks, and the birth was classified as a miscarriage.

If these 76 children are included, we find 3.7 % instead of the previously calculated 3.3 % "born dead" of all children born at public clinics during the period — not a very great difference.

According to information in the case records, there were 37 live born children who were counted as residents of Oslo in the reports but who did not belong in this group. Moreover case records were lacking for 7 children for whom birth reports had been sent in.

Table 3 includes the children whose mothers were undoubtedly residents of Oslo and whose case records were present in the public clinics.

In this table and hereafter in this article, babies will be spoken of as born alive or still-born, and the designation

Table 3. *Children born at public clinics in Oslo 1930—39.*

Birth weight	Total	Born alive		Stillborn	
		n	%	n	%
More than 2500 g	15836	15579	98.2	257	1.6
Up to 2500	1127	893	79.2	234	20.8
Total	16963	16472	97.0	491	2.9
% permatures	6.7	5.4		47.7	

Table 4. *Sex distribution for full term and premature infants born at public clinics in Oslo 1930—39.*

	Birth weight over 2500 g					Birth weight 2500—400 g					Sex unknown
	Total	Boys		Girls		Total of known sex	Boys		Girls		
		n	%	n	%		n	%	n	%	
Born alive .	15579	8084	51.9	7495	48.1	890	401	45.1	489	54.9	3
Died within 24 hours..	52	32	61.5	20	38.5	85	43	50.6	42	49.4	3
Stillborn ...	257	140	54.5	117	45.5	225	124	55.1	101	44.9	9
Total	15836	8224	51.9	7612	48.1	1115	525	47.1	590	52.9	12

"born dead" as used in the birth reports will not be employed, since children who died during the first 24 hours after birth are included among the live born.

The table shows that according to birth weights there were 6.7 % prematures among all of the babies born at public clinics in Oslo during 1930—39. This figure is considerably higher than that obtained on the information in the birth reports on the duration of pregnancy. Among live born children there were 5.4 % prematures as compared to 47.7 % among the still-born.

Table 4 shows the sex distribution for the full term and premature babies. Among the full term babies (birth weight over 2500 gm) there were 51.9 % male and 48.1 % female. The percentage of male infants was somewhat higher among the still-born than among the live born.

Among the premature babies there was a difference between the still-born and those born alive. Among the still-born there was a majority of males, 55.1 % as compared with 44.9 % females, while of those born alive there were 45.1 % males and 54.9 % females. The reason why there are more female babies than male in the last group might be that the former weigh, on the average, less than the latter and thus more of them will be under 2500 gm. Consequently more premature females than males grow up. This agrees well with the findings of other authors (Capper, Maurzey).

Table 5. *Premature infants born in Oslo 1930—39 classified according to birth weight.*

Birth weight	Total prematures		Born alive		In % of all in the weight group	Stillborn		In % of all in the weight group
	n	%	n	%		n	%	
400—1000 gr.	74	6.5	11	1.3	15.0	63	27.0	85.0
1001—1500 gr.	144	12.7	89	9.8	71.9	55	23.3	38.1
1501—2000 gr.	258	22.8	201	22.6	88.0	57	24.4	22.0
2001—2500 gr.	639	56.5	592	66.3	92.6	47	20.1	7.4
Not weighed ..	12	1.5	0	0	0	12	5.1	100.0
Total	1127	100.0	893	100.0	79.3	234	100.1	20.7

Among the prematures were 12 babies whose sex was not recorded in the case record. These children were still-born or died during the first 24 hours. According to information on the duration of pregnancy and other information, it is practically certain that their weights were between 2500—400 gm (some of them were not weighed).

The premature babies were distributed according to birth weight as shown in Table 5.

Of the premature babies born alive, the majority belong to the highest weight classes. Over 66 % had a birth weight over 2000 gm and about 89 % weighed more than 1500 gm.

The premature still-borns were more evenly distributed in the different weight classes, but are most frequent in the lowest weight class.

Discussion.

The figures for the frequency of premature births depend on the limit drawn up between premature birth and miscarriage, and on whether the calculation is based on all children born during the period or only on those born alive.

In Table 6 the frequency of prematurity is calculated on total births where nothing else is stated. The limit towards miscarriage varies, but no authors have put it lower than 400 gm.

Table 6. *Frequency of premature births in various investigations.*

Author	Locality	Year	% premature of total number born	% premature of live births
Sunde	Oslo	1908—22		7.3
Brander	Helsinki	1919—27	12.8	
Räihä	Helsinki	1927—37	11.3	
		1942—46	6—7	
Crosse	Birmingham mat. home	1931—43	8.9	
»	Birmingham	1943	6.3	5.4
[1]Lichtenstein	Stockholm	1936—38	6.2	
[1]V. Sydow	Göteborg	1940	5.0	
Tysen	Philadelphia	1930—44	9.4	
Maurzey	Chicago	1925—39	7—8	
Taylor et al.	Colorado	1946—49	13.2	
Boesen	København	1942—46	5.0	
	Roskilde	1935—44		
Blegen	Oslo	1930—39	6.7	5.4

[1] Quoted from Räihä

The table shows that the frequency of premature births is lower in Oslo than most other places during the same period, except for Stockholm and Roskilde. After 1940 lower figures have been found in Gothenburg and figures about the same as Oslo in Helsinki og Birmingham.

The figures from America are, on the whole, somewhat higher than the corresponding European ones. This may be due to the fact that the American material includes a good many negroes. Some authors have claimed that prematurity is more frequent among negroes than among whites. Others, on the contrary, find that prematurity is just as frequent among whites from a low social level.

It must be assumed that the decrease in frequency of premature births in Oslo from 1908—22 to 1930—39 is due to improvement in the general social conditions.

The weight distribution in the different materials varies considerably. This is significant in evaluating mortality. Table 7 presents the weight distribution in some of the materials discussed here.

Table 7. *Weight distribution of prematures born alive.*

Author	1000 g and less	1001—1500 g	1501—2000 g	2001—2500 g
Ylppö (668 infants)	The 3 groups together 68 %			32.0 %
Sunde (1423 infants)	0.4 %	8.9 %	24.9 %	65.8 »
Tyson (1977 infants)	19.8 »	16.7 »	20.9 »	42.9 »
Blegen (893 infants)	1.3 »	9.8 »	22.6 »	66.5 »

The weight distribution in my material agrees with that found by Sunde. In the American material a much larger proportion of the premature babies belong to the lowest weight classes, and relatively few to the highest.

In Ylppö's material only a relatively small proportion of the children are in the highest weight class.

VI. THE CAUSES OF PREMATURE BIRTHS

The factors usually considered as causes or contributory causes of prematurity are:

1. Multiple births (twins or triplets).
2. The mother's health during pregnancy, possible pathological conditions (of the mother, fætus or placenta).
3. Age of mother at delivery.
4. Birth order of the child.
5. The social position of the mother (legitimate or illegitimate baby, financial state, housing conditions etc.).

These factors are examined in the following on the basis of the material collected.

1. Multiple births. Table 8 shows the frequency of multiple births among the premature babies born at the public clinics, among the full term babies born at the public clinics, and among all children born in Oslo during the same period. The table shows that there is a great excess of multiple births among the prematures. There are about 12 times as many multiple births among the prematures as among the full term babies born at the public clinics. If the calculations are

Table 8. *Distribution of multiple births among premature and full term infants born at public clinics, and among all births in Oslo 1930—39.*

	All births			Live birth			Stillbirth		
	n	multiple	%	n	multiple	%	n	multiple	%
Public clinics Premature	1127	212	18.8	893	180	19.4	234	32	13.7
Public clinics Full term	15836	237	1.6	15579	227	1.5	259	10	3.9
Oslo Bureau of Public Health	23611	602	2.6	22961	559	2.6	650	43	6.6

made only on those born alive, the difference is even greater — namely 19.4 and 1.5 % respectively.

These figures are in good agreement with those found by other investigators. Räihä and Brander found that multiple births comprised more than 20 % of the prematures, Crosse found 16.6 %, Sunde more than 14 % and Maurzey about 13 %.

There has been discussion as to whether twin and triplet pregnancy as such may be a cause of premature birth, but there is also the possibility of an indirect predisposing cause such as toxæmia or uterine hæmorrhage. Moreover, it must be remembered that the definition of prematurity is based on birth weight, and this artificial limitation may be too inclusive, so that babies with a lower weight than 2500 gm may not always be really premature.

Table 9 shows that only for 108 of 212 premature twin or triplet children, multiple birth is the only known cause of prematurity. They comprise about 10 % of all prematures. As for the remainder, about half, the multiple birth is combined with toxæmia of varying degree or uterine hæmorrhage.

2. *Pathological conditions, maternal, placental, fœtal.* Table 9 is compiled from the information in the case records. All pregnancies where there has been any albuminuria, raised

Table 9. *Causes of premature birth.*

Causes	Stillborn		Live births		Total prematures	
	n	%	n	%	n	%
Maternal:						
a. Toxæmia	66	28.2	218	24.4	284	25.2
b. Syphilis	9	3.9	2	0.2	11	1.0
c. Pulmonary disease, dis. of urinary tract, and others	7	3.0	58	6.5	65	5.8
Fætal:						
a. Malformations, hydramnion	23	9.9	8	0.9	31	2.7
b. Intrauterine death	44	18.8	0	0.0	44	3.9
c. Multiple birth with no other cause	5	2.1	103	11.5	108	9.6
Placental:						
a. Placenta prev.	11	4.7	11	1.2	22	1.9
b. Abruption placenta	20	8.5	27	3.0	47	4.2
c. Deep placenta — hæmorrhage	16	6.8	67	7.5	83	7.4
Unknown:						
a. Healthy during pregnancy		14.1	388	43.5	421	37.4
b. No information	0		11	1.2	11	1.0
Total	234	100.0	893	99.9	1127	100.1

blood pressure and threatening or manifest eclampsia of pregnancy have been classified as toxæmia. Even if other factors have complicated the pregnancy or delivery, such as multiple birth, hæmorrhages, disease of the mother, the pregnancy is classified in the group "toxæmia" if any of the above-mentioned symptoms were present.

Where the placenta appeared to be the most important cause of premature birth, however, the case is included in the latter group even if albuminuria or multiple birth is a complicating factor. Multiple birth is considered the cause only where nothing pathological is found in connection with the pregnancy or delivery.

Table 10. *Frequency of the various causes of premature births.*

Causes of premature birth	Number of premature infants	
Unknown cause	432	38.4
Toxæmia	284	25.2
Placental causes sole.....	152	13.5
Multiple birth as cause....	108	9.6
Other maternal causes	76	6.7
Other fætal causes	75	6.6
Total	1127	100.0

Table 10 presents a summary of the sequence of these causes of prematurity as regards frequency. The table shows that in 432 cases of 1127, i. e. in 38 %, no cause of prematurity could be demonstrated.

The largest group is toxæmia which comprises 25.2 %, but toxæmia as the sole cause is found in only 19.8 %.

Placental causes also comprise a large group. Here, too, there is a combination of several causes in some cases.

Multiple births as the sole cause are found in 9.6 %, and are thus quite frequent. This comprises about half of all multiple births (Table 8). In the rest of the multiple births there were other causes also.

My figures for the individual causes agree well with those found by others. It should be noted, however, that syphilis is very rare in my material (1.0 %). For comparison it can be mentioned that Sunde found syphilis in 6 % and Maurzey in 4 %. Crosse found syphilis in only 0.6 %.

3. Mother's age at delivery. Table 11 shows the mother's age at delivery. It appears that the largest percentage of premature babies are born to mothers aged 25—34 years.

Among the still-born the figures are shifted to a higher age, as relatively more are born to mothers aged 35—45 years, and fewer to mothers under 25. This distribution of

Table 11. *Age of mother at delivery.*

Age of mother in years	All prematures		Born alive		Stillborn	
	n	%	n	%	n	%
Under 20 years	51	4.5	42	4.7	9	3.9
20—24 »	207	18.3	170	19.2	37	15.8
25—29 »	336	29.7	280	31.3	56	23.9
30—34 »	317	28.3	251	28.1	66	28.2
35—39 »	168	14.9	124	13.8	44	18.8
40—44 »	38	3.4	22	2.5	16	6.8
45 or more	3	0.3	2	0.2	1	0.4
Age unknown	7	0.6	2	0.2	5	2.1
Total	1127	100.0	893	100.0	234	99.9

the various age groups corresponds, on the whole, to the usual age distribution of mothers at delivery in Norway.

Among the unmarried mothers there is, as is expected, an excess of young mothers. However the age distribution of the unmarried mothers of premature babies is in complete agreement with the age distribution for unmarried mothers in towns all over the country (Fig. 1).

4. The birth order of the child. Table 12 shows that most of the premature babies are the first born, both as regards babies born alive and still-born. Far along in the birth order, as number 4 or more, still-borns are more numerous than those born alive. This may be due to a higher frequency of pathological conditions in the mothers in the higher age

Table 12. *Birth order of the premature infants (Relative figures).*

	Total infants	Birth order			4 or more	Unknown	Total
		1	2	3			
Born alive	893	67.4	19.6	6.5	6.6	-	100.1
Stillborn	234	61.0	20.2	6.4	12.0	0.4	100.0
Total	1127	66.1	19.7	6.5	7.7	-	100.0

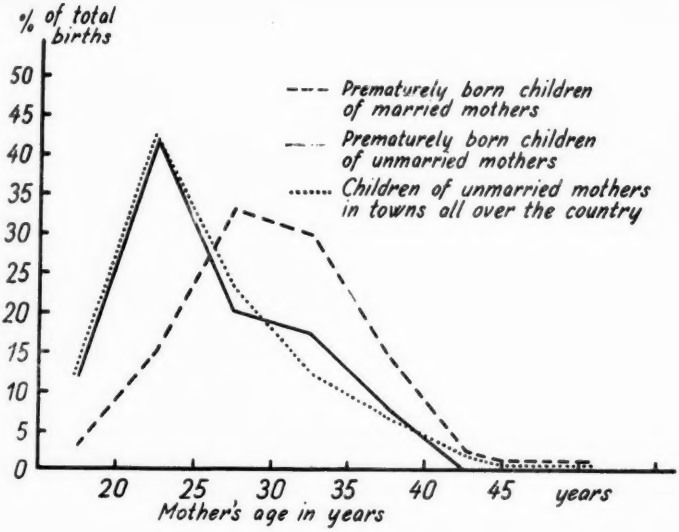

Fig. 1.

groups. For the 2nd and 3rd children born to mothers, the situation is the same for those born alive and still-born.

Table 13 shows a comparison between the distribution of prematures in birth order and The Bureau of Vital Statistics' records on all births in towns in Norway during 1933—39. As the records of the bureau only include ligitimate births, only prematures born to married mothers are included in this comparison. The bureau's figures include only children

Table 13. *Percentual distribution of birth order of infants born alive in cities in Norway 1933—39, and of prematures born at public clinics in Oslo 1930—39. Legitimate births.*

	Total n. born	Infants' birth order			4 or more	Total
		1	2	3		
Cities in Norway	60739	50.0	26.2	11.4	12.4	100.0
Prematures at public clinics .	685	66.0	21.3	6.3	6.4	100.0

born alive, and as children who die during the first 24 hours are not included here, these have not been included among the prematures either.

Thus it appears that among the premature babies there are more who are first born than there are first borns in a material of ordinary births. The difference is even greater than shown in the table, as the figures for towns in Norway also include premature children.

5. Social conditions. Social conditions include a number of different factors. It was difficult to obtain accurate information on several of these, such as the financial position of the family.

The factors on which there is adequate information and which may serve to elucidate the problem, are whether the birth is legitimate or illegitimate, and housing conditions. As regards the first factor, it may safely be assumed that the unmarried mothers on the average have lived under poorer conditions than the married ones.

As regards living conditions, it seems justified to consider them as a certain indication of the social conditions in the period of the investigation.

Marital status of the mother.

Of the 1127 premature babies born at the public clinics, 953 are legitimate and 174 illegitimate. Unfortunately there is no accurate information as to how many of the entire material of 16 963 children born at public clinics in Oslo in the period covered by the investigation are legitimate and illegitimate, but the figure may be approximated by using the number of illegitimate births from Oslo's Bureau of Public Health's birth reports.

During the period 1930—39 these were 8 % of all births. If we assume that the same holds good for the material from the public clinics, we find that 1357 of the 16 963 children were illegitimate. The frequency of premature births to married and unmarried mothers calculated in this manner appears in Table 14.

Table 14. *Frequency of premature births to married and unmarried mothers.*

	Total	Premature	
		n	%
Legitimate	15606	953	6.1
Illegitimate	1357	174	12.8
Total.....	16963	1127	6.6

This calculation shows that premature births are twice as frequent among unmarried mothers as married ones.

The objections which might be raised against this calculation, e. g. that there are perhaps more unmarried mothers who are delivered at home than at the clinics, are compensated by the fact that there are certainly very few unmarried mothers among those delivered at private clinics. In addition to this, all the mothers from the institutions for mothers are delivered at the public clinics, and most of these are unmarried. The figures presented here should thus be justified.

Housing conditions.

Table 15 shows the distribution of the prematures according to housing conditions. The classification in housing groups has been made by grouping the births according to the parish in which the mother lived at the time the child was born. The parishes are divided into 3 classes according to population density so that Class I includes the parishes where the population density during 1933—36 was an average of 100 persons per 100 rooms, Class II 150 persons per 100 rooms, and Class III 204 persons per 100 rooms. Class I includes the parishes: Frogner, Uranienborg and Fagerborg. Class II: Markus, Gl. Aker, Trefoldighet, Vår Frelser, Hauge, Gamlebyen, Jacob, Matteus, Paulus, and Class III: Vålerengen, Grønland, Lilleborg, Kampen, Petrus, Sagene and Torshov. Further details of this classification may be

Table 15. *The premature births distributed according to population density in the parishes where the mothers resided at delivery.*

Population density	Total born in Oslo 1930—39	n prematures	% prematures
I	4850	167	3.5
II	8893	428	4.8
III	9869	504	5.1
Unknown		28	
Total	23611	1127	4.8

found in Axel Strøm's publication "De akutte smittsomme sykdommer og boligene".

The same increase in premature births corresponding to poor housing conditions was also found if only legitimate births were used as a basis for calculation.

More detailed information was obtained by direct questioning as regards housing at the time of the child's birth in the case of 505 children. On this basis a classification into groups has been made according to the amount of crowding in the child's home (Table 16). The groups are the same as those employed in the official housing statistics.

Table 16 shows that there was more crowding in the homes of the premature babies than that which corresponded to the distribution of the population of Oslo in the various housing groups during the same period. While only 1.2 % of the population of Oslo in 1934 lived in "extremely overpopulated apartments", about 15 % of the prematures lived under such poor conditions, and in the "overpopulated" group the difference was also marked, namely 5.4 % and 27.3 % respectively.

It may be assumed that the difference was even greater than these figures show, as most of those who did not have their own homes but lived with relatives certainly lived under very poor conditions.

Even though the material available for evaluating the significance of social conditions for premature births is

Table 16. *Living conditions for 505 prematures during period after birth.*

Living conditions	n children	In % of total	Distribution of Oslo's population Dec. 1, 1934
Very roomy	0	0	5.5
Roomy (One person per room or less)	74	14.7	56.4
Densely populated (Max. 2 persons per room)	147	29.1	31.5
Crowded (Max. 3 persons per room)	138	27.3	5.4
Very crowded (More than 3 persons per room)	74	14.7	1.2
No home: (Live with relatives)	49	9.7	
Child removed to childrens home, home for mothers, hospital, etc.	23	4.6	
Total	505	100.1	100.0

limited, the results presented indicate that the frequency of premature births increases as living conditions become poorer. This is in good agreement with the findings of others. For example, Baird found the following figures for the frequency of premature births in 3 different population groups: Among mothers from the working class 9 %, among mothers from the upper and middle classes 6 %, and among mothers who had consulted a specialist 2 %. Crosse found premature births about twice as frequent among poor mothers as among those better situated, and Tyson found that among 750 pregnant women who lived on a diet rich in proteins, vitamins and minerals and consulted a physician regularly, there were no premature births, while in a corresponding control material in which the mothers diet was not regulated, there were 37 premature births, 5 %.

He claims that mothers who live on a proper diet will not only give birth to fewer premature babies, but among the children born before term there will be more in the weight class 2500—2000 gm and fewer with a weight under 1000 gm. Other investigations along the same lines can be mentioned:

Ebbs et al. (1941), Burke et al. (1943) and K. Utheim Toverud (1945).

It is reasonable to assume that it is usually a combination of these factors which lead to premature births.

Poor finance are often the cause of bad housing, limit the opportunity of obtaining medical advice during pregnancy, and often do not allow of a proper diet during pregnancy. Improper diet is believed to be a contributory cause of toxæmia.

Age certainly plays some part among the unmarried mothers, and these are often primiparae.

Poor social and economic conditions thus definitely afford an increased risk of premature birth.

VII. MORTALITY AMONG PREMATURE CHILDREN

It appears from the above that still-births are much more frequent among prematures than among full term infants. (See Table 3.) Table 17 shows that the same is true of mortality during the first 24 hours after birth. For comparison the figures from the birth reports to the Oslo Bureau of Public Health are also presented. In evaluating them it must be remembered that they included both full term and prematures. The table shows that still-births are 13 times (9 if only prematures over 1000 gm are included) as frequent among prematures as among full term infants. Among the live births, mortality during the first 24 hours is 33 times as high for prematures as for full term infants.

The influence of the mother's marital status is presented in Table 18. According to this table it does not seem that the unfavorable social conditions under which the unmarried mothers have lived has had any significant influence on the frequency of still-births or mortality during the first 24 hours of life. This may be due to the fact that the majority of the unmarried mothers are much younger than the married ones when the first child is born. It is also possible that a difference in mortality in premature babies born to married or unmarried mothers would not be demon-

Table 17. *Frequency of stillbirths and deaths within 24 hours among infants born at public clinics in Oslo (full term and premature) and total for Oslo 1930—39.*

	Total born	Born alive		Stillborn		Died within 24 hrs.	
		n	%	n	%	n	% of live births
Premature, public clinics ..	1127	893	79.2	234	20.8	88	9.9
Full term, public clinics ...	15836	15579	98.2	257	1.6	52	0.3
Total Oslo	23611	22961	97.2	650	2.8	207	0.9

strable until after discharge from the clinics. This will usually be about 4 weeks after delivery so that any eventual difference will not be apparent from a comparison of the neonatal mortality in the 2 groups.

Table 19 shows the distribution of mortality among the prematures during the first year of life. For comparison there are only the figures from the Bureau of Public Health for Oslo for the first 24 hours and for the first year.

The table shows that the mortality among the prematures is much more concentrated within the period immediately following birth than among infants in the whole city.

Table 20 shows mortality among the prematures up to the age of 9 years. No calculations for children over 9 have been made, because the information available is from 1948 when the youngest prematures in the present material were 9 years old (born in 1939. Two premature children were

Table 18. *Frequency of stillbirths and deaths within 24 hours among prematures born to married and unmarried mothers.*

Prematures at public clinics 1930—39	Total born	Stillborn		Born alive		Died within 24 hrs.	
		n	%	n	%	n	% of live births
Legitimate	953	197	20.7	756	79.3	70	9.3
Illegitimate.....	174	37	21.3	137	78.7	18	13.2
Total	1127	234	20.8	893	79.2	88	9.9

Table 19. *Mortality during first year.*

Died	Premature born at public clinics 1930—39		Total C. o 1930—39	
	n	%	n	%
Within 24 hrs.	88	46.3	207	26.1
2nd—7th day.....	50	26.4		
2nd—4th week	16	8.4	586	73.9
2nd—12th month	36	18.9		
Total 1st year.....	190	100.0	793	100.0

more than 9 when they died.) The figures from the Oslo Bureau of Public Health for the whole city are used for comparison.

The table shows that the life expectancy of the prematures is much shorter than for children in the city as a whole, mortality is 5 times as great for the first 8 years of life taken as a unit. It appears that the difference in mortality is greatest during the first year of life, subsequently it is less pronounced. The difference is, in reality, somewhat greater than the table shows, as the figures from the Bureau of Public Health also include the premature children.

Table 20. *Mortality before age of 9 years for prematures born at public clinics 1930—39 and for Oslo as a whole (relative figures).**

	Oslo	Prematures	%
Born alive	22961	893	
Died during 1st year in %	3.5		22.20
—»— 2nd » » »	0.7		1.10
—»— 3rd—4th year in %	0.4		1.12
—»— 5th—8th » » »	0.5		0.84
Total died before age of 9 in %.	4.9		26.2

* The relative figures are calculated on the basis of those alive at the beginning of each year. For the prematures the calculation is based on those on whom there was information at the beginning of the year.

Table 21. *The influence of birth weight on mortality among the prematures. Relative figures.*

Birth weight	Prematures born alive	Died within:				Died:		Total death 8 first years
		24 hours	1 week	1 month	1 year	2nd year	3—9 years	
1000 g or less	11	54.5	90.9	100.0	100.0	0.0	0.0	100.0
1001—1500 g	89	27.0	53.9	60.7	69.7	0.0	0.0	72.0
1501—2000 g	201	17.4	22.9	24.9	31.1	2.3	4.2	37.2
2001—2500 g	592	3.9	5.7	6.7	10.0	0.8	1.5	13.1
Total	893	9.9	15.5	17.3	22.2	1.1	2.0	26.2

Birth weight is a significant factor for the survival of the prematures, as shown in Table 21. It appears from the table that about 74 % of the premature children on whom there is information were alive in 1948 and had reached school age. Mortality is highest in the lowest weight classes and decreases with increasing weight. None of the children in the lowest weight class lived for more than a month. In the weight group 1001—1500 gm about ¼ were alive after 8 years, and of the weight group 1501—2000 gm about 60 %, while in the highest weight group about 85 % were alive after 8 years. After the children are a year old, birth weight no longer seems to have any significance on mortality.

Table 22. *Mortality during the first 8 years for legitimate and illegitimate prematures.*

	Total born alive	Died within:				Died:					
		24 hours		1 month		2.—12th month		1st year		2nd year	3—9 years
		n	%	n	%	n	%	n	%		
Legitimate	756	70	9.3	125	16.6	25	4.0	150	20.2	0.5	2.2
Illegitimate ...	137	18	13.2	29	21.8	11	13.2	40	35.7	5.8	0.0
Total	893	88	9.9	154	17.3	36	5.1	190	22.2	1.1	2.0

Table 23. *Mortality before 9th year among single and multiple premature births.*

Prematures Public clinics 1930—39	Born alive n	Died before age of 9 years	
		n	% *
Single births	713	165	25.8
Multiple births	180	44	27.5
Total	893	209	26.2

* The percentage calculated is based on the total number of children on whom information was obtained.

In Table 22, mortality for prematures born to married and unmarried mothers is compared. The greatest difference in mortality is found in the 2nd to 12th month of life, i. e. after discharge from the clinics, and during the 2nd year of life. There are so few prematures who died after the 2nd year of life that the calculations made for the period after 2 years of age do not mean much, but the table may indicate that after the children are 2 years old there is no increased mortality for the illegitimate prematures.

Table 23 shows mortality up to the age of 9 years for prematures born singly compared with multiple births. There is no difference between the two groups. In this material 19 % of the children are multiple births (one set of triplets).

See Table 9 for the causes of still-births among the premature children.

The causes of death for the premature children who were born alive are presented in Table 24. Here are also included the 2 premature children who died after the age of 9 years.

The table shows that congenital debility and cerebral and meningeal hæmorrhage dominate during the first month. After the first year these causes of death have no significance. The children who die from malformations also die during the first few weeks. On the other hand we find that infectious diseases are the cause of most deaths among the older children. The most important of these are infections in the respiratory tract, bronchi and lungs.

Table 24. *Mortality for 211 prematures, born in Oslo 1930—39.*

Cause of death	Neonatal death	Died in 2nd—12th month	Died after 1 year	Total mortality
Congenital Debility	92	11	-	103
Hæmorrhagia cerebri et mening.	32	-	1	33
Bronchitis, pneumonia	2	13	9	24
Malformations	13	2	-	15
Intrauterine O_2 insufficiency	10	-	-	10
Cramps (Anuria)	2	3	-	5
Accidents	-	1	3	4
Gastroenteritis	2	1	-	3
Other infections (tetanus, meningitis)	-	1	1	2
Tumors (cerebral, sacral) .	-	-	2	2
Mors subita	1	1	-	2
Tuberculosis	-	1	-	1
Syphilis	-	1	-	1
Nephritis	-	-	1	1
Unknown cause. No information	-	1	4	5
Total	154	36	21	211

Delivery was artificially induced in only 2 of the 33 cases where the cause of death was cerebral hæmorrhage. For the remaining 31 children where the delivery was spontaneous, nothing pathological was demonstrated in 14, either regarding pregnancy, the placenta or delivery, but 4 of the children were twins. In 13 cases there was a pathological condition of the mother (toxæmia), and in 2 cases the delivery was complicated (breech or foot presentation), and forceps were used in 2 cases.

Discussion.

The investigation shows that the premature children had an excessive mortality during the period involved. Stillbirths were about 13 times as frequent among prematures as among full term infants, and about 33 times as many prematures died during the first 24 hours after birth. The increased mortality risk persisted during the first years of

Table 25. *Stillbirths, neonatal mortality and "total mortality" in Oslo compared with some American and English figures.*

Author	Locality	Period	Premature total	Stillborn %	Neonatal mortality %	Total mortality %
Tyson	Philadelphia	1930—44	2960	17.6	20.9	38.5
Maurzey	Chicago	1925—39	819	12.5	13.9 (2 weeks)	
Crosse	Birmingham	1943	1268	17.3	22.9	40.2
»	Birmingham mat. home.	1931—43		10.5	11.4	21.9
Blegen	Oslo	1930—39	1127	20.8	13.8	34.6

life so that after 8 years about 5 times as many prematures as full term children had died.

Table 25 is presented for comparison, showing figures for some English and American cities. The table shows still-births and neonatal mortality separately and combined (total mortality).

As seen from the table, the figures vary considerably for both still-births and neonatal mortality. This is partly due to the fact that different limits have been applied for what is considered a miscarriage. In Birmingham the limit is a pregnancy of 28 weeks' duration, while in most of the other series the limit goes to a lower birth weight (down to 400 gm).

Compared with the other figures, the Oslo material has a relatively high percentage of still-births and a low neonatal mortality.

In my material there was no difference in the frequency of premature still-births to married and unmarried mothers. But among the prematures born alive mortality was higher among the illegitimate than among the legitimate children, at least up to the age of 2 years.

Most of the foreign investigations have demonstrated that both still-birth frequency and neonatal mortality are higher among illegitimate than among legitimate children. Since no such difference was demonstrable for the still-birth fre-

quency in our material, it may be due to the fact that we were not able to compare groups which were identical as regards age of mother, birth order of the child, etc.

The great significance of birth weight for survival expectancy is clearly apparent from Table 21. The figures in Table 26 may be used for comparison.

The table shows that the situation is pretty much the same, and the variations which exist may be due to different opportunities for special care at the different clinics.

VIII. THE MENTAL DEVELOPMENT OF THE PREMATURES

A. Distribution among different schools.

Of the 893 premature children who were born alive, 211 died as mentioned in the preceding chapter. Of the surviving 682 children there were, as mentioned on p. 12, 141 on whom no further information was obtained for various reasons. There thus remained 541 children.

Table 27 shows the sex and weight distribution of these children.

If this weight distribution is compared with the weight distribution of all premature born alive (Table 21) it is seen that a majority among the survivors are in the higher weight groups and fewer are in the lowest, as is to be expected.

Table 28 shows the distribution of these children in different kinds of schools which they either have attended or are attending.

It would have been desirable to test all of these children for intelligence. However there was no opportunity of doing this, and the mental development of the children cannot, therefore, be evaluated on that basis. However there is some information which may serve to elucidate the problem.

Children who attend the primary schools in Oslo are usually tested by their teachers in the first grade. These measurements alone are not of great value as the tests are group tests and are performed by a number of different

Table 26. *Neonatal mortality among prematures.*

Birth weight	Crosse	Tyson	Maurzey (under 2 weeks)	Taylor et al.	Blegen
1000 g or less	100.0	98.0	93.3	91.0	100.0
1001—1500 g	55.5	80.0	81.7	38.0	60.7
1501—2000 g	20.5	54.0	32.9	15.0	24.9
2001—2500 g	3.4	19.0	9.1	3.0	6.6
Total	12.8	-	26.3	16.0	17.2

teachers. But in combination with the teacher's impression of the child during the earliest school years, they do afford a basis for an evaluation. Those with a low test and who have difficulties scolastically and in their adjustment, are sent to the school psychiatrist after examination by the school physician and the school's specialists in ophthalmology and otorhinolaryngology. Thus it can be assumed that almost all children who are mentally retarded to any significant degree will be detected.

All the intelligence tests in this material were made by the school psychiatrist, Dr. Augusta Rasmussen, with the exception of one which was carried out at Sophies Minde (school for cripples).

Of the 476 children who are registered as pupils in the primary schools — of which 198 were boys and 278 girls — 18 were tested. One of these had an I. Q. of 64. This was a pupil who was not tested until the 6th grade because she had attended an out of town school. It was considered too late to move her over to the special school. There were 8

Table 27. *Sex and weight distribution of 541 prematures.*

Birth weight	Boys	Girls	Both sexes	
			n	%
1001—1500 g	9	11	20	3.7
1501—2000 g	47	58	105	19.4
2001—2500 g	174	242	416	76.9
Total	230	311	541	100.0

Table 28. *Distribution of 541 premature children in schools and institutions.*

Birth weight	Total	Ordinary primary schools Oslo and Aker	Private — out of town schools	Special schools	School for cripples	Corrective school	Feeble school	
1001—1500 g	20	18	2	-	-	-	-	-
1501—2000 g	105	80	8	9	3	-	2	3
2000—2500 g	416	376	11	21	1	3	2	-
Total	541	476	21	30	4	3	4	3

with an I. Q. between 71—80, while 9 had an I. Q. of 81—90. There is no information on the other 458, but there is reason to believe that they had an I. Q. of over 80.

Of the 21 who are registered as pupils of *private schools or out of town schools,* there are 4 who were tested and should have been transferred to the special school (I. Q. 71—80) but whose parents sent them to private schools to avoid the special school. Information obtained from the parents about 2 others indicates that they were mentally retarded.

Of the 30 children who attend or have attended a *special school* there are 2 who suffered diseases at an early age which may cause mental injury. One had meningitis at the age of 13 months. She is a twin and the other twin has normal intelligence. Before she was stricken there was little difference between them, but since then the one who has had meningitis has been retarded both mentally and physically when compared with the other twin.

In the other case the child had a head injury when 18 months old which was serious enough to send him to a hospital with the diagnosis: Cerebral concussion. According to the mother he was a normally lively and intelligent child before the injury, but since then he has been rather dull and his memory has been bad.

In addition to these 2 it has been stated that one child has a congenital heart defect which has retarded its development.

It should be noticed that for 20 of the other 27 children who attended the special school there was information of nervous disease in the family such as insanity, epilepsy, alcoholism, amorality of one of the parents and furthermore, poor housing, sickness or death of one parent so that the social and economic situation at home was difficult.

Of these 30 there were 14 with an I. Q. 51—70, 13 had 71—80 and only 3 had 81—90.

Of the 4 who are registered as pupils of Sophie Minde's School for Cripples (boarding school), one had normal intelligence while 3 had an I. Q. between 51—70. Two of these had Little's disease while the 3rd had been retarded physically and mentally after poliomyelitis at the age of 4 ½. The one who was normal was treated at the institution for an extreme thoracic kyphosis.

The 3 children who attended *corrective schools* were all retarded with an I. Q. between 71—80. It should be noted that among the children who attended regular primary school and who are registered among the 476 above, 2 came to corrective schools after the conclusion of primary schooling because of their abnormal behaviour. These two were mentally on the borderline for special school pupils. In all these 5 cases the parents had an anti-social attitude and the authorities had been forced to step in.

For the 4 children who attended or still attend *Torshov School for Feeble minded* there is no more detailed information, but as the children are not up to special school level they probably have an I. Q. under 60.

The 3 children who are institutionalized at *Tokerud Home for Mental Defectives are all idiots* (I. Q. under about 35). One of them is the 3rd of the only set of triplets in the material. She suffers from hypothyroidism. The child was born at the Oslo Municipal Maternity Clinic and was transferred from there to Dept. VIII at Oslo City Hospital and afterwards to the home for mental defectives. One of the

Table 29. *Intellectual grouping according to I. Q. (Brander's classification.)*

Schools and institutions	I. Q. 35 Idiots	I. Q. $\leqslant$ 35—50 Imbeciles	I. Q. 51—70 Debile	I. Q. 71—80 Bordering on feeble minded ness	I. Q. 81—90 Physiologically stupid	I. Q. $\geqslant$ 90 Normal and superior
Feeble minded institution	3	-	-	-	-	-
Feeble minded school ..	-	4	-	-	-	-
School for cripples	-	-	3	-	-	1
Corrective schools	-	-	-	2	1	-
Private schools	-	-	-	4	2	10
Out of town schools ...	-	-	-	-	-	5
Special school	-	-	14	13	3	-
Ordinary primary school	-	-	1	8	9	458
Total	3	4	18	27	15	476
Boys	2	2	10	11	8	199
Girls	1	2	8	16	7	275

other idiots has had epileptic attacks since he was 1 month old, and came to Heftye's Children's Home when he was 2 months old, and thence at the age of 1 year to the institution for mental defectives. The third is extremely defective and cannot talk. He came to the institution when he was 6 ½ years old. He had previously been at the Åkebergveien Home for Infants from the age of about 4 months.

Table 29 is a compilation of the information collected. The reservations which must be taken in regard to this table, especially the column to the right, have been stated above.

The table shows that there are 25 children who must be classified as feeble minded (14 boys and 11 girls) with an I. Q. of 70 or below. The percent of feebleminded is thus 4.6 % for both sexes. For boys alone it is 6.1 % and for girls alone 3.5 %.

The sex distribution of all children with an I. Q. under 90 shows 33 of 230 boys or 14.7 % and 34 of 311 girls or 10.9 %.

The frequency of special school pupils among the prematures.

It appears from the above that 30 of the premature children attend or have attended special school. In 2 of these the retarded intellectual development may have been due to other causes. If we exclude these, we have 28 of 541 children 5.2 %. But in addition to these 28 children there were 2 who attended regular primary school who should have been at special school. (8 others were doubtful with I. Q. 71—80 but after examination by the school psychiatrist it was recommended that they continue at regular school.) There were also 4 pupils in private schools, 3 in corrective schools and 3 in school for cripples who were at the intellectual level of special school children. Altogether this makes 40 pupils of 534 who were originally sent to ordinary primary schools or 7.5 %. There were 24 boys and 16 girls.

Thus among 226 boys there were 24 special school pupils or 10.6 % and of 308 girls there were 16 special school pupils or 5.2 %. The excess of boys is significant.

For comparison it can be mentioned that according to information obtained from the office of the special school, the number of special school pupils in Oslo from 1930—37—38 was about 2.8 % of all school children in Oslo. After 1938—39 this figure increased somewhat and reached a peak in 1943 with 3.8 %. Since then it has decreased and at the beginning of 1946—47 it was 3 %.

For the school year 1946—47 there were 3.2 % special school pupils among all the school children at public primary schools in Oslo. That same year there were 5.4 % special school pupils among the premature children in the corresponding schools, thus a considerable excess of special school pupils among the premature.

In the annual report of the Oslo Public Schools it is seen that the majority of the pupils in special school come from schools in the districts with the poorest housing. It has been mentioned previously (p. 28) that on the average housing conditions were poor for the premature children, and a large number of these belong to the school districts

Table 30. *Further schooling after primary school for 249 premature children.*

	Completed primary school Oslo and Aker	Completed other primary schools
No further schooling	30	16
Continuation	92	6 (of which 4 from special school)
Higher school	102	3
Total	224	25

from which the largest number of special school pupils are recruited. Thus, the fact that there are more special school pupils among the prematures than among other school children may be due to housing and living conditions just as much as to their prematurity per se.

During the same school year the ages of the premature children did not deviate much from the normal age in the different classes of the regular Oslo primary schools (Annual Report of Oslo Public Schools).

In 1949 there were 224 of the premature children who had attended and finished regular primary schooling. The same was true of 25 of the children who had attended out of town schools, private schools, special schools and Sophie Mindes boarding school. Table 30 shows the further schooling of these pupils.

B. Intellectual development judged on the basis of scholastic achievement.

As mentioned on p. 13 information was obtained from the teachers for the 476 premature children who had attended regular school as to the childrens' scholastic achievement and the teachers' personal impression of the children.

This information was obtained from forms, similar to those in use in Oslo since 1947 for occupational guidance of children who have completed their primary schooling, filled out by the teachers. Usually the form is filled out by the Form Master or Mistress who knows the child well.

Table 31. *Scholastic achievement in scholastic and practical subjects for premature children from different social groups (both sexes).*

Social groups	Above average		Average		Below average		Total n marks
	n	%	n	%	%	%	
Gr. I. Roomy living conditions	105	27.8	203	53.7	70	18.5	378
Gr. II. Densely populated	213	28.4	416	55.4	122	16.1	751
Gr. III. Crowded	130	19.8	361	55.0	165	25.2	656
Gr. IV. Very crowded	61	17.1	192	54.0	103	28.9	356
Total	509	23.8	1172	54.7	460	21.5	214

The form includes marks in a number of subjects, and in addition, columns where the teacher may characterize the pupil as "above average, average and below average". For those pupils who have completed their primary schooling the final marks have been used, for the others the results of their latest examinations. On the whole there was good agreement between the school marks and the opinion of the teacher. In the cases where the marks and the opinion of the teacher did not agree, the opinion of the teacher was given most weight. In the calculations the mark T has been taken as average, anything better as above average and anything lower as below average. On the whole this agrees well with the personal opinion of the teachers.

The form also has columns where the teacher can give his evaluation of the children's speed at work, thoroughness, concentration and persistence, independence, initiative, leadership qualities, cooperation and mental stability.

In making calculations on this information the 3 middle columns have been taken as average, the column farthest to the left as below average (— variants) and most to the right as above average (+ variants).

Table 32. *Scholastic achievement in scholastic subjects for prematures from different social groups (both sexes).*

Social groups	Above average		Average		Below average		Total n marks
	n	%	n	%	n	%	
I	64	31.8	97	48.3	40	19.9	201
II	116	28.2	217	52.7	79	19.2	412
III..	68	19.5	167	48 0	113	32.5	348
IV.....	30	15.6	95	49.5	67	34.9	192
Total	278	24.1	576	50.0	299	25.9	1153

First a comparison was made of the scholastic achievement of prematurely born school children from 4 different social classes, judged according to housing conditions (Table 31). The figures in the table were obtained by counting each subject separately (distributed among the groups average, above average, and below average) and then adding the results for all the subjects. Only those children on whom there was definite information as to housing conditions are included in the table. These are 387 of the 476 children.

It appears from the table that scholastic achievement decreases with poorer housing conditions (social conditions).

As a child may have very different capabilities in scholastic and in practical subjects, the scholastic achievement in the scholastic subjects is compared in Table 32 for the same premature children. It is seen that the relation between poor social conditions and scholastic achievement is even more pronounced than in the preceding table.

Table 33, shows the distribution of those in the different social groups who had to repeat classes and confirms this relationship. For comparison some figures from B. Ribsskogs book: "Repeaters in the Primary School", are presented. He has calculated the number of repeaters for the 10 year period 1930—1940 for all the primary schools in Oslo. He has divided the schools in 3 groups, from good, fair and poor housing districts in the city, and he shows that there are most repeaters in the schools where the

Table 33. *Distribution of repeaters among prematures from different social groups.*

Social groups	Total n pupils	Repeaters	
		n	%
I.	68	1	1.5
II.	140	9	6.4
III.	116	12	6.3
IV.	66	5	7.6
Living conditions unknown	86	10	13.2
Total	476	37	7.8

children come from the poorest social and economic homes. The average figures for the 3 groups of schools in this period were:

Boys [1]	R: 5.5 %	8.9 %	10.5 %
Girls	R: 3.7 %	6.1 %	7.4 %
Total	R: 4.6 %	7.5 %	9.0 %

These figures are practically the same as those found for the premature children during the period 1937—49.

In the same manner as for scholastic achievement, a comparison has been made of the teachers' impression of the children (lower part of the form) for the 4 social groups. The counts were made in the same manner as for scholastic achievement and, as mentioned above, the children are classed as + variants, average, and — variants. The result is presented in Table 34 and it is seen that the situation is the same as for scholastic achievement; a decreasing number of + variants and an increasing number of — variants as the social conditions grow worse.

The results of what has been presented above show that if the intelligence of premature children is to be compared with ordinary school children on the basis of scholastic

[1] R: the percentage of all repeaters of the total number of pupils during a year.

Table 34. *Teachers' opinions of premature pupils from different social groups (both sexes).*

Social groups	+ variants n evaluations	%	Average n evaluations	%	— variants n evaluations	%	Total n evaluations
I.....	79	18.3	311	72.2	41	9.5	431
II	134	16 3	647	78.7	41	5.0	822
III.....	89	11.9	577	77.0	83	11.1	749
IV.....	38	9.1	316	76.0	62	14.9	416
Total.....	340	14.1	1851	76.6	227	9.4	2418

achievement, it is necessary to compare children from the same social level if the results are to have any value.

In order to obtain adequate material for comparison the following procedure has been followed:

From 1947 to 1950 a total of 150 premature children had completed their primary schooling (60 boys and 90 girls). From the register of the Occupational Guidance Office a corresponding number of girls and boys were selected from the same schools and the same classes. The selection was made by going through the cards in alphabetical order so that the children were chosen at random. In order to insure that the control material was really adequate, the occupations of the fathers in the 2 groups were checked, and very good agreement was found.

Table 35 shows a comparison between the scholastic achievement of the 150 premature children and that of the control group. The results are given in percent of all of the evaluations in each subject.

It appears from the table that the premature children are practically like the other children in practical subjects and drawing. This is true of both boys and girls, but among the premature boys there is, perhaps, a wider range with more above and more below average than in the control material.

If the practical subjects (sewing, wood and metal work) and drawing are taken as a whole, it is found that the

Table 35. *Scholastic achievement for premature children and other school children from the same social milieu in Oslo 1946—47 to 1949—50 (relative figures).*

	Above average		Average		Below average	
	Prem.	Control	Prem.	Control	Prem.	Control
60 boys						
Scholastic subjects ...	13.6	21.7	56.8	54 8	29.7	23.5
Practical » ...	21.7	15 0	58.3	68.3	20.0	16.7
Drawing	18.3	15.0	55.0	61.7	26.7	23.3
90 girls						
Scholastic subjects ...	18.0	22.6	47.1	47.6	35.0	29.8
Practical » ...	25.7	26.4	54.9	58.2	19.5	15.5
Drawing	30.0	22.9	60.0	59.0	10.0	18.1
Both sexes (150 children)						
Scholastic subjects ...	16.4	22.3	50.6	50.2	33.0	27.6
Practical » ...	24.3	22.4	56.1	61.8	19.7	15.9
Drawing	25.3	19.6	58.0	60.1	16.7	20.3
Practical subjects and drawing	25.4	21.1	57.0	61.0	18.3	17.9

situation is very similar for premature children and others, and *not* any poorer.

For the scholastic subjects the situation is different. Among the boys there is a marked difference between the prematures and the controls, in favour of the latter. The difference is less pronounced for the girls, but here, too, the control material has the advantage. For both boys and girls the group designated "average" is almost equally represented by the prematures and the controls.

The results thus show that among the children who attend regular primary schools there are just about as many average children among the prematures as among other children, but it seems that there are, among the prematures, a larger number under average and fewer over average than in the control material, and that this is more pronounced for boys than for girls.

In the following (Table 36) a comparison is made between scholastic achievement of premature children with different birth weights. The 18 premature children with a birth weight of 1500 gm or less are compared with the 150 premature

Table 36. *Comparison between scholastic achievement of prematures with birth weight 1500 g or less (A) and total prematures (B). Both sexes. Relative figures.*

	Above average		Average		Below average	
	A	B	A	B	A	B
Scholastic subjects ...	16.7	16.4	53.7	50.6	29.5	33.0
Practical subjects and drawing	15.7	25.4	68.7	57.0	16.6	18 3

children discussed above, the large majority of which have a higher birth weight. Among the 18 small premature children is included one with Little's disease who really should have attended special school. The table shows no parallelism between birth weight and intellectual development.

The lowest birth weight among these 18 children was 1200 gm, and she was average in all subjects.

In the annual report from the Oslo schools for the school years 1946—47 and 1947—48 the examination marks have been calculated in arithmetic and Norwegian (also English) for the pupils who have completed their primary schooling. The marks have definite values. S: 5, Mg: 4, T: 3, Ng: 2 and Lg: 1. Calculations have been made for boys and girls separately, and the means and mean errors are presented. The corresponding values have been calculated for the prematures who completed primary schooling during the same 2 years.

The numbers are small, 9 boys and 13 girls from 1946—47 and 16 boys and 18 girls from 1947—48. Table 37 shows the result of this comparison. The table shows that in no case is there a statistically significant difference between the figures for the prematures and the entire material, but there is an average tendency for the figures of the prematures to be slightly lower than the figures of the entire material. In the figures from the Oslo schools the premature children are also included, but they represent such a small fraction of the entire material that this does not have to be considered. The last class at the primary schools these 2 years consisted of 1659 and 1709 pupils respectively.

Table 37. *Examination marks for the premature children in comparison with the average for the Oslo primary schools in 1946—47 and 1947—48.*

Sucject	Boys		Girls	
	Oslo schools	Premature	Oslo schools	Premature
1946/47				
Norwegian	2.85±0.029	2.67±0.118	3.15±0.026	3.00±0.200
Arithmetic	2.90±0.040	2.33±0.333	2.73±0.037	2.55±0.116
1947/48				
Norwegian	2.84±0.032	2.81±0.118	3.20±0.027	3.06±0.640
Arithmetic	2.97±0.036	3.06±0.233	3.15±0.037	2.72±0.292

Discussion.

Previous investigations of the mental development of premature children show highly varying results.

This may be due to the differences in the materials treated. The children may have been examined at a different age, or the birth weight may have varied considerably, and above all, the social conditions under which the children have grown up have not been uniform.

Intelligence tests have been used regularly as a basis for evaluating intelligence.

The earliest publications, as mentioned in the introduction, showed that a large number of the premature children were mentally retarded. The most pessimistic view of the prognosis for premature children, and especially for those with a birth weight of 1500 gm or less, was stated by Capper in his conclusion: "In brief, the immature infant will become the backward schoolchild, is the potential future psychopathic or neuropathic patient and even the potential inmate of the home for imbeciles and idiots." Both Ylppö and Capper made their investigations in the period after the First World War, in Berlin and Vienna respectively, and the social environment of the children examined was very bad. The poor prognosis which these authors made for premature children may be due largely to the poor conditions under which the children examined had grown up.

Table 38. *Frequency of various anomalies in 541 premature children.*

Anomalies	n	
Eye anomalies		
Faulty refraction	68	
Strabismus	22	
Color blindness	10	
		100
Poor hearing		2
Other central nervous		
Stuttering	7	
Faulty speech	1	
Word blindness	2	
Enuresis	10	
Tic	2	
Spasms, epilepsi	9	
Lefthandedness	11	
Little's disease	3	
Pareses of other origin	2	
		47
Hernia		
Operated	16	
Unoperated	4	
Spontaneous recession	21	
		41
Cryptorchism		2
Other defects		
Congenital heart disease	5	
Defects in columna and joints	4	
Diabetes	1	
Acetonemic vomiting	1	
Anomalies of the urether	1	
Infantilism	1	
		13
Total		205

Similarly, the poor prognosis made by Salomonsen after the examination of premature infants admitted to Oslo City Hospital, Dept. VIII, may be explained by the fact that only the most debile and sickly children were transferred there from the Oslo Municipal Maternity Clinic.

The difference in prognosis as regards mental development for prematures of different weight classes is found repeatedly in the publications of several authors. Brander found a considerable difference in the mental development of children with a birth weight over and under 2000 gm (11.2 % and 28.6 % respectively with I. Q. 70 or less). He also found that in addition to a higher frequency of feeble mindedness, there was in general a lower mental development in prematures than in other children. Girls were mentally somewhat superior to boys. It was obvious that the majority of the prematures which he had examined came from the lowest social level of the population, and in a great many of them he found hereditary taints (with alcoholism and feeble mindedness as the most predominant).

In the 1930's Hess and his collaborators found a different result: ⅖ths of the premature children examined were of normal intelligence, ⅖ths were above average, and ⅕ths were above average, and ⅕th lower.

In more recent publications, as mentioned on p. 9, it is claimed that the prognosis for the premature (both physical and mental) is largely dependent on the care the children receive, and the conditions under which they grow up.

Hedwig Koenig thus found no statistically significant difference in mental development between premature and ordinary school children from the same environment (I. Q. measurements). Neither did she find any striking difference between prematures with high and low birth weights.

A comparison of the results reported in the literature and the present results reveals the following:

The results from the Oslo material are in part considerably better in respect to the mental development of premature children than would have been expected from a study of earlier investigations, but not quite so good as claimed by some more recent authors.

It should be emphasized that the scholastic achievements of the prematures were, on the whole, only slightly less than those of ordinary children. The girls did somewhat better than the boys.

On the other hand, the influence of the children's social background was striking.

It seems that the difference in intelligence between prematures and ordinary children could be reduced if the living conditions of the prematures were improved.

It was found that the *small* prematures were about the same as the rest as regards scholastic achievement. It should be noted that none of the premature children with a birth weight of 1500 gm or less who grew up were classified as feeble minded, and only a few were under average intelligence.

The fact that a larger proportion of prematures than of ordinary children were transferred to special schools may largely be due to the social background of the prematures, which was worse than that of the average school child in Oslo. This might also possibly be changed by an improvement in the living conditions of the prematures.

It was found that 4.6 % were feeble minded, i. e. I. Q. 70 or less. For comparison: Brander (1936) 11.2 % and Koenig (1950) 2%. Even though better control of pregnant mothers and improved gynæcological assistance might reduce the number of feeble minded prematures, it will still be necessary to expect a larger number of feeble minded among these children than among full term babies because of their greater susceptibility to trauma during delivery. There are, nevertheless, not so many of them as the earliest publications seemed to imply.

IX. THE PHYSICAL DEVELOPMENT OF THE PREMATURE CHILD

A. Height and weight.

The school's height and weight measurements recorded on the school health cards are used as a basis for evaluating the physical development of the premature children.

The average height and weight of the premature children in the different age groups has been compared with the averages for school boys and girls in Oslo. Seven-year-olds

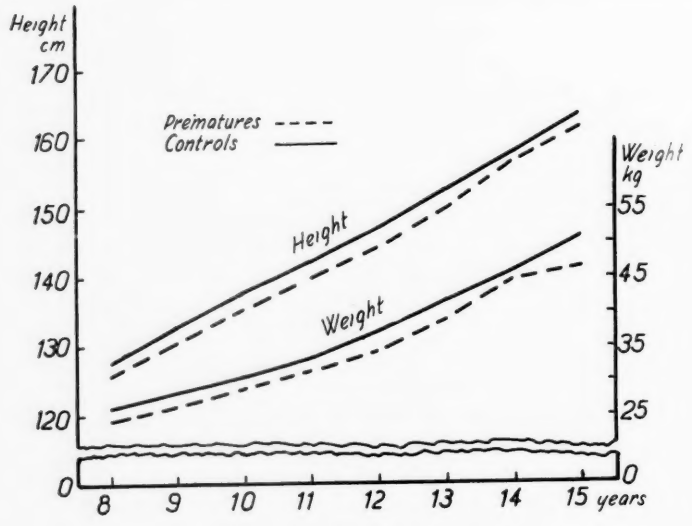

Fig. 2. Height and weight.
Prematurely born boys and boys in Oslo primary schools.

are not included as the tables are based on weighings in March, and most of the 7 year age group (6 ½—7 ½ year) was only measured and weighed at the beginning of the school year in the autumn.

Figs. 2 and 3 show the average height and weight in the various age groups for the girls and boys in the Oslo primary schools during the period 1938—47, and for premature children during the same period.

It appears from the figures that the average height of both premature boys and girls is lower than for ordinary school children in Oslo. The average weight is also lower the whole time for premature boys than for ordinary school boys, but for the girls there is a compensation at the age of 12 years.

To determine whether the poor social conditions under which the premature children live might be the cause of the height-weight difference, a comparison has been made be-

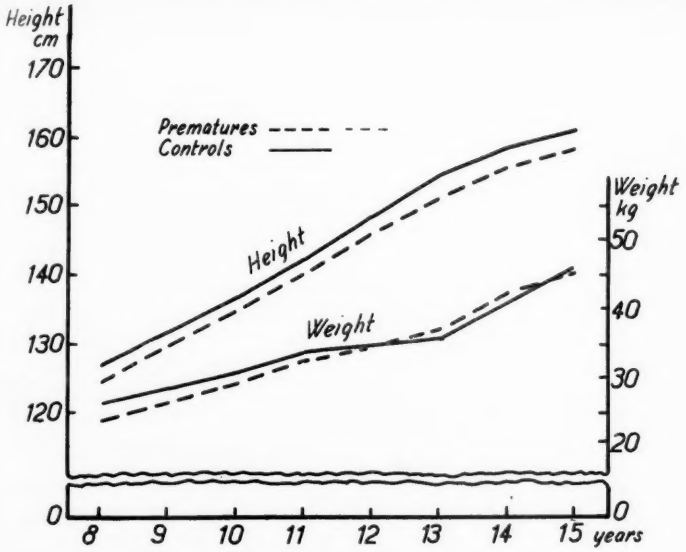

Fig. 3. Height and weight.
Prematurely born girls and girls in Oslo primary schools.

tween the average height and weight of the 150 premature children and the controls described on p. 47. Here the 7 year olds are included, where the figures in both materials are taken from the physical examination of the first grade in the autumn, just after they have started school.

Figs. 4 and 5 show that the result is unchanged in respect to height, but as regards weight it is even more obvious that the prematures are slightly under the normal average.

There is no tendency to compensation of either height or weight toward puberty for either sex. The difference between the individual groups is *not* statistically significant, but it can be stated that there is probably a real difference because all of the figures in the entire series are lower for the prematures than for ordinary school children.

A comparison of the weight/height relationship for the 150 prematures and their controls reveals the following: (see the curves in Fig. 6).

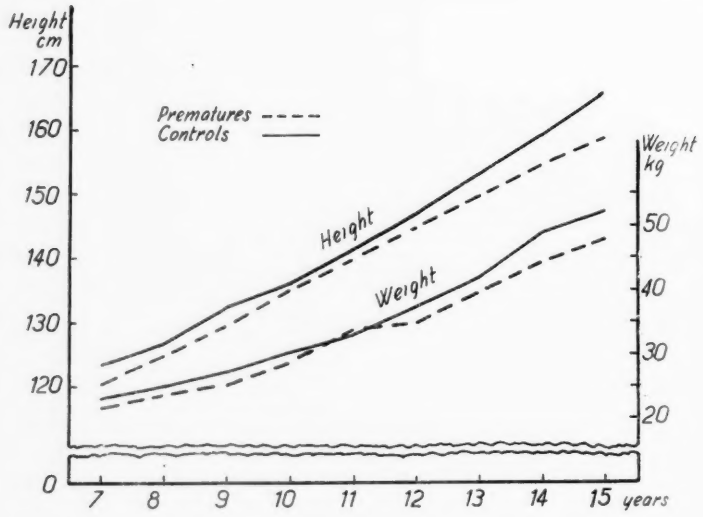

Fig. 4. Height and weight. Prematurely born boys and controls.

For the boys it seems that the prematures are, on the whole, shorter and lighter, but otherwise fairly well proportioned. For the girls, on the other hand, it is found that the weight/height curve is higher than that of the normal material, indicating that they are relatively overweight.

B. Malformations, defects and nervous manifestations.

Among the 525 children on whom more detailed information was obtained by direct questioning, and a total of 541 when supplemented by the health cards etc., there were relatively many with various defects. Table 38 presents these in detail. Some of the children had several anomalies so that the total number of defective children is somewhat less than the number of defects in the table.

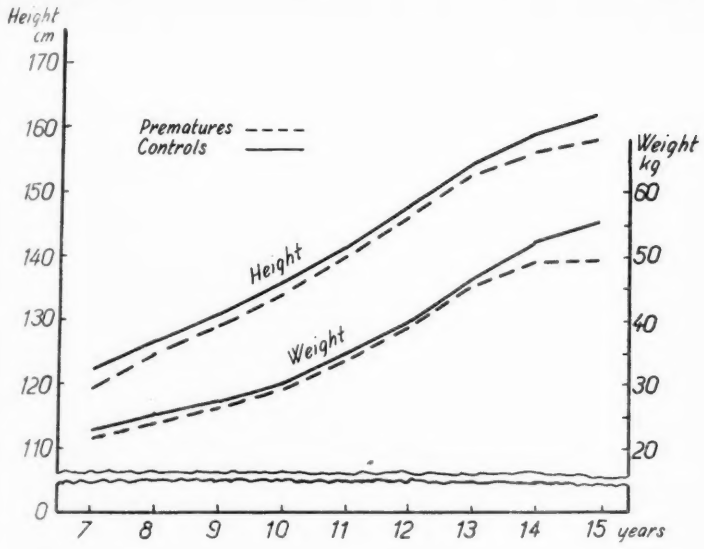

Fig. 5. Height and weight. Prematurely born girls and controls.

It should be noted that among the children with eye anomalies, there were no cases of retrolental fibroplasia.

From the school health cards and from the children's mothers it has been learned that 256 of 541 premature children have had their tonsils and adenoids removed.

Little's disease.

There are 4 cases of Little's disease among 851 premature children who were followed until the age of 9 years, or 0.4 %. Two of these had attended boarding school at an institution for cripples and one had attended regular primary school. The 2 former were "feeble minded" with I. Q. 70 or less, and the 3rd had an I. Q. of 76, which is on the border of feeble mindedness.

In addition to those 3 it has been ascertained that one child who died of bronchopneumonia at the age of 8 also

had Little's disease. The mental level of this child is not known.

As the diagnosis is often not made until after the child has started to walk, there is a possibility that there may have been some undiagnosed cases among the children who died earlier, or among those on whom no information has been obtained after the age of one year, so that the percentage might be somewhat higher.

According to the modern conception, the value of ordinary intelligence tests for children with Little's disease is doubtful, so the intelligence of the above described children may be somewhat higher than has been calculated here.

Discussion.

Earlier investigations on the physical development of premature children have also yielded highly variable results. Some authors have claimed that the prematures will always be inferior to the full term children in both height and weight, and that those with the lowest birth weight will lag farthest behind (Capper, Brander). Others are of the opinion that development is only delayed, and that the differences are evened out at puberty (Ylppö), and finally there are some who claim that there is no real difference after the first 2 years of life (Koenig).

The cause of the variability of these results may be that the basis for comparison has not been uniform.

Ylppö's investigation (1919) included 700 premature children of which 68 % weighed less than 2000 gm at birth. He found that most of them had attained normal height and weight by the age of 5—6 years, only some of the very smallest (birth weight under 1000 gm) remained under average in height and weight after they had grown up.

V. Sydow (1936) and Brander (1938—41) found that the premature were under average in physical development. The age of the children when examined was 3—9 years and 0—7 years respectively. Hess (1934) and Schultze (1939) found no difference in height and weight when they used sibs as

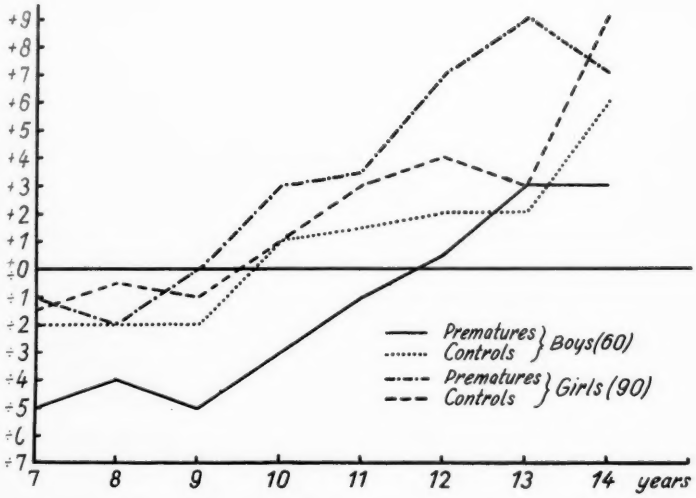

Fig. 6. Weight/height relationship for 150 prematurely born pupils and their controls.

controls. Levine and Gordon (1942) also found no striking difference between premature and other children from the same social environment. Drillien (1948) found that the average height and weight was lower for premature children than for a standard material (Holt and McIntosh's standard for normal children from 1940). The difference was greatest for the children with the lowest birth weight, and the difference was greater for weight than for height. The author mentions that it is probable that the same unfavorable economic and social conditions which were responsible for the premature birth continued to have an influence on the child and its development.

Sunde (1930) showed that the premature children were considerably less in height and weight than Oslo primary school pupils, but no consideration was taken of differences in social background.

Koenig (1950), on examination of 650 premature children, found that they did not deviate from the average in height

and weight after the first 2 years of life, and there was no significant difference for children with varying birth weights (150 children had a birth weight under 2000 gm). The age of the children examined varied from ½—6 years.

The present investigation of premature children in Oslo born during the period 1930—39 and followed up to the age of 14—15 years, has shown that the average height and weight of these children is below the average of ordinary school children in Oslo.

This may be due to the fact that most of the premature children are from school districts where the social and economic level of the population is generally low. Carl Schiøtz has demonstrated, in an investigation of the physical development of school children in different primary schools in Oslo, that the average height and weight for children from the schools in the financially best situated districts are higher than for children from districts with a poorer social background.

As shown by Fig. 4 and 5, there is still a difference between premature school children and others in average height and weight when the social difference is eliminated. The premature school children are somewhat below ordinary school children in both height and weight, and even though the difference is not large, it is encountered in all age groups.

This difference is not large enough to be of any significance for the future of the premature children. There is a possibility that the premature children in Oslo would have made a better showing if they had had the same special care as the American children studied by Koenig.

On the other hand, a larger number of the small prematures will have a chance to grow up with special care according to the latest American figures (Helen Reardon, James L. Wilson and Bruce Graham) where it is claimed that in the weight class 2001—2500 gm at least 93 % will reach the age of one year, in the class 1501—2000 gm at least 82 % and in the class 1001—1500 gm at least 50 %, while the corresponding figures in the present series are 89.5 %, 64.3 % and 25.3 %.

This increased survival rate might possibly have an influence in the opposite direction. As there are only 20 children with a birth weight under 1500 gm who reached school age in the Oslo material, it has not been possible to make any satisfactory comparison between the average height and weight of these and others with higher birth weight.

Table 38 shows that the most frequent defects encountered in the premature children in the Oslo material are sensory anomalies, especially of the eyes, and other central nervous affections such as stammering, enuresis, etc. In regard to the latter, it is probable that it is more frequent than stated, as parents often are ashamed to admit that the child is so affected. In addition to these defects, hernia was relatively frequent.

This is in good agreement with the findings of other authors. Of the relatively large number of premature children with eye anomalies, there were none in the Oslo material of such a severe nature that there was any danger of blindness.

Hypertrophic tonsils or adenoids were frequent, in 46 %, but as I have not been able to obtain information as to how frequently tonsils and adenoids were removed in other children during the same period I cannot state whether this frequencey is higher than normal.

Little's disease was found in 0.4 % of the Oslo material, while Sunde found 0.7 %, Ylppö 3.1 % and Capper 5 %. The two last mentioned probably found much higher figures because the average birth weight in their material was lower than in the Norwegian ones.

X. SUMMARY AND CONCLUSION

1. The frequency of premature births in Oslo during the period 1930—39. Of all the children born to mothers resident in Oslo at public clinics here during the above-mentioned period, 6.7 % were premature (cf. Table 3). Of all children born alive, the prematures comprised 5.4 % as compared to

47.7 % of still-births. During the corresponding period there were only a couple of cities in Sweden and Denmark (Stockholm and Roskilde) where the figures were equally low (cf. Table 6). This result is found in spite of the fact that the lowest limit toward miscarriage in the Oslo material is 400 gm. This limit is lower than that generally used in Europe and corresponds to the lowest American limit.

The sex distribution among prematures in Oslo corresponds to that usually found, namely more females than males.

Over half of the premature children in Oslo had a birth weight of 2001—2500 gm (56.5 %), while only about 20 % fall in the weight groups 1500 gm or less. The weight distribution of prematures born alive shows an even greater excess for the weight class 2001—2500 gm.

A similar distribution was found earlier in Oslo by Sunde, but in publications from other localities relatively more prematures are found in the lowest weight classes (cf. Table 7).

2. *The causes of premature birth.* In 38 % of the cases it was not possible to demonstrate the cause of the premature birth. Maternal causes were found in 32 %. Toxæmia was the largest group (syphilis was found in only 1 %). Placental causes and multiple births followed in frequency (cf. Tables 9 and 10). Of the 1127 premature children, 18.8 % were twins or triplets, but in only 9.6 % was the multiple birth the only known cause of prematurity. In the other cases the multiple births were combined with toxæmia or placental complications.

Among the full term children born in Oslo in the same clinics during the same period, only 1.6 % were twins or triplets.

These figures are in good agreement with those found elsewhere.

It has further been demonstrated that a larger number of premature children were first born than in a normal material (cf. Table 13). There were relatively more mothers of premature children under 20 and over 30 years of age than mothers of mature children, i. e. premature birth was rela-

tively frequent in very young mothers and in primiparae over 30.

Premature birhts were almost twice as frequent among unmarried mothers as among married ones. It was also found that prematurity was more frequent under poor living conditions than under adequate ones (cf. Tables 14, 15 and 16).

Age is certainly an important factor for the unmarried mothers, as well as the fact that most of them are primiparae.

The relation between the age of the mother, the birth order of the child and premature birth otherwise agrees with the literature.

The influence of social factors also agrees with more recent American and English publications. In America, particularly, the significance of diet for the frequency of prematurity has been emphasised. It is claimed that proper diet during pregnancy will not only reduce the number of premature, but will especially reduce the number of prematures with very low birth weights.

There are relatively fewer prematures in Oslo than are reported from American and English cities, and fewer prematures with very low birth weight, and this is probably because slums are practicaly non-existant in Oslo. Those who are classified in the lowest social classes have, nevertheless, in most cases had a fairly good diet. All have had the opportunity of free medical examination at control antenatal clinics where they also obtained advice as to diet, etc.

3. Still-births and mortality. In about 21 % of all premature births, the infants were still-born, while the corresponding figure for full term deliveries was 1.6 %, i. e. still-births were about 13 times as frequent in premature as in full term births (cf. Table 17). On the other hand there was no difference in the frequency of still-births for married and unmarried mothers (cf. Table 18).

The higher still-birth frequency among prematures is in agreement with previous reports. But a *uniform* stil-birth frequency for married and unmarried mothers does not

agree with more recent investigations from England and Scotland. There it has been found that the still-birth frequency is twice as high when the mothers have lived under poor conditions as under satisfactory ones. It may be assumed that the majority of unmarried mothers live under difficult social conditions. Since the still-birth frequency in the present investigation was not greater for unmarried than for married mothers, this may be because the unmarried mothers were on the whole, younger than the married ones, and also because the difference in standard of living is not as great in Norway as in England and Scotland. Moreover there was readily accessible free medical advice during pregnancy, and medical assistance at delivery was the same for both groups.

It was demonstrated that the risk of death was much greater for a premature infant than from those from a normal pregnancy (cf. Table 20). The difference was greatest during the first year of life, later it was less pronounced. During the first year of life the neonatal period was the most critical. Of the premature children who died during the first year, 81 % died in the neonatal period (46.3 % during the first 24 hours).

Of all prematures born alive, 9.9 % died during the first 24 hours, while the corresponding figure for a normal series was 0.9 %.

Mortality was greatest among the prematures with a low birth weight, so that no premature infant with a birth weight of 1000 gm or less lived more than a month (cf. Table 21).

There was some excess of mortality among premature children whose mothers came from a poor social background (unmarried mothers), and this was most marked in the period after discharge from the clinic, during the child's first year, but was less pronounced subsequently (cf. Tab. 22).

A good many of the causes of death of the prematures were directly related to prematurity. In these cases the diagnosis was "congenital debility". The next most frequent causes were hæmorrhages of the brain and of the cerebral membranes. After the first year of life infections were the most frequent cause of death (cf. Table 24).

An increased frequency of still-births and "total mortality" was also demonstrated where the mother had complications during pregnancy or delivery.

These results on the frequency of still-births and mortality are in general agreement with earlier findings. The higher still-births frequency in the Oslo material as compared with some English publications is due to the fact that the latter use a limit of 28 weeks above miscarriage, while the present material includes prematures with a birth weight down to 400 gm.

There is no great difference in neonatal mortality between the Oslo material and American and English figures, but in the lower weight groups the mortality is a little higher in Oslo. It is reasonable to assume that this difference is due to varying opportunities for special care of prematures (cf. Tables 25 and 26). It is particularly likely that the deaths classified under the diagnosis "congenital debility" could be reduced considerably by special care and trained personnel.

The still-birth frequency was lower for premature twin and triplet children than for single children, but the mortality was the same.

4. The mental development of prematures. Of 541 premature children on whom information was obtained through 1948—49 when the control examination was made, there were at least 4.6 % with an I. Q. of 70 or lower.

Of the 534 premature children who were registered at ordinary primary schools, 7.5 % were special school pupils. There were relatively more prematures among the pupils of the special schools than among the pupils of the Oslo primary schools in the school year 1946—47. The majority of the prematures were from the poorest residential districts from which the largest number of the special school pupils were recruited.

The was no marked deviation in age for the premature children as compared to the normal age in the classes in the ordinary primary school.

There was a marked correlation between the social background of the premature children, rated according to hous-

ing conditions, and their scholastic achievement and character (cf. Tables 31, 32 and 34). The percentage of repeaters was decidedly less among prematures from the best social background than among those who lived under poor housing conditions (cf. Table 38). In the school year 1946—47, however, there was no marked difference in the percentage of repeaters between prematures and ordinary school children.

When the scholastic achievement and personal character of the prematures was compared with a control material from a corresponding social background, a slight difference was found. This difference was in favour of the control material. The scholastic achievement of the premature girls was somewhat better than that of the boys (cf. Table 35). There was no pronounced difference in mental development for the prematures with the lowest and highest birth weights. None of the prematures with a birth weight of 1500 gm or less could be classified as feeble minded.

Compared with reports in the literature, this shows that the mental development of the premature children in the Oslo material is considerably better than earlier investigations have indicated. Some more recent investigations from America show even more favorable results. This improvement is probably due to the better opportunities for special care during the earliest period, and continued control for the American prematures, all the way up to school age.

5. *The physical development of prematures.* The average height and weight for premature children in Oslo, born during the period 1930—39, are somewhat lower than for ordinary school children in Oslo during the same period. The oldest children were followed until they were 14—15 years old (cf. Figs. 2 and 3). When social differences are eliminated by comparing premature children with others from as similar a background as possible, the prematures still had a lower height and weight than other school children, and there was no tendency to compensation toward puberty (cf. Fig. 4 and 5). The difference, however, was so slight that the figures were within the normal variation in the individual age classes.

6. Malformations, defects and nervous manifestations in premature children. The distribution of various anomalies in prematures in Oslo was about the same as that found elsewhere. Eye anomalies, nervous manifestations and hernia were the most frequent. There were no cases of retrolental fibroplasia. Little's disease was found in 0.4 %, while previous authors have reported considerably higher frequency. This may be connected with the weight distribution in the different materials.

7. Conclusion. All the evidence indicates that good living conditions during pregnancy and medical control throughout the entire period reduce both the number of premature births and cause relatively more of the prematures born to be viable. It is, therefore, of the greatest importance, to continue to establish and extend the activity of control clinics for pregnant women, and see to it that they are instructed on proper diet and the way of living. It would be possible to reduce mortality among the prematures by having specially trained nurses at the maternity clinics to care for these premature children. A single clinic could be a centre where prematures from the entire district received care, as has been done in America. Moreover, it must be regarded as certain that the continued care of premature infants after discharge from the clinic, especially during the first 2—3 years, as regards diet and hygiene, is of basic significance for their future mental and physical development. If special control clinics could be established for these children, where there were also possibilities for helping the needy, the difference between full term and premature children later in life would probably be reduced considerably.

This holds for premature children who have not suffered injury at birth which has caused irreparable harm. However this occurs so rarely that no efforts should be spared to ensure a minimum mortality among premature children.

APPENDIX

Occupational Guidance ..... Date .....

Confidential

Pupil's first name ..... Surname .....

Born ..... Class ..... School .....

1. School Marks:

	Over average	Average	Below average
a) Written Norwegian			
b) Oral Norwegian			
c) Arithmetic			
d) Woodwork/sewing			
e) Drawing			
f) Physical training			

2. Other remarks on school work:

a) Has the pupil special interest for one or more particular subjects?
b) Has the pupil special talents for languages? Yes/No.
c) Has the pupil special talents for mathematics? Yes/No.
d) What significant variations have there been in the pupils scholastic achievement? (Examples of answer: "Gradual decline since 5th grade", "Considerable progress the last year".)
e) Has the pupil had to repeat any class? Yes/No. Which?

3. Information on the Pupil:

If you do not feel competent to answer any of the following questions, they may be left unanswered. (The following questions should be answered by checking the column most suitable.)

a) Speed at work:

Very slow.	Slow when not encouraged.	Not very fast.	Fast when encouraged.	Very fast.

b) Thoroughness:

Very careless.	Careless when not watched.	Not very thorough.	Can be thorough.	Very thorough.

c) Concentration and persistence:

Scatter-brained, gives up easily.	Eager, but tires quickly	Works fairly steadily.	Can be persistent.	Good concentration.

d) Independence:

Helpless alone.	Works badly alone.	Likes to have support.	Can work independently.	Works best independently.

e) Initiative:

Never takes initiative.	Works best under direction.	Has ideas, but rarely puts them into practice.	Can take initiative.	Full of ideas and initiative.

f) Leadership ability:

Never takes the lead.	Does not like to take lead.	Can lead, but seldom does.	Often takes lead.	Pronounced leadership.

g) Co-operation:

Cannot co-operate.	Poor co-operation.	Not flexible in co-operation.	Can co-operate.	Co-operates readily.

h) Mental stability:

Unbalanced, labile.	Easily upset.	Somewhat variable.	Well balanced.	Very stable and calm.

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DETERMINATIONS OF STANDARD ENERGY METABOLISM (BASAL METABOLISM) IN NORMAL INFANTS

by

Petter Karlberg

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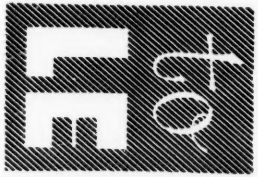

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PREFACE

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Appendix

Corrections:

Page	Line	*At present:*	*Change to:*
15	6—7	... principle *and it seems to have been* ...	... principle, *which was* ...
19	16	the air *density* of the system ...	the air *tightness* of the system ...
21	14	... the chamber oxygen *back*	... the chamber oxygen *is*
28	Table 1.	(in heading) O_2 CO_2	O_2 *error* CO_2 *error*
		(in column 2.) Mean *error*	Mean
		Range *of error*	Range
30	1	Malm. In 1948, Malm demonstrated ...	Malm. In 1949, Malm demonstrated ...
43	Fig. 11.		*R = rectal temp. Dp = dew point AT, AL, Sp = air temp. at the top and bottom of resp. chamber, and in the spirometer.*
45	7	shown in Fig *11.*	shown in Fig. *8.*
49	14	The correlation between ...	The uniformity between ...
50	Fig. 14 and Fig. 13.		*AT, AL, Sp = air temp. at the top and bottom of resp. chamber, and in the spirometer.*
55	8	to *question* the term ...	to *introduce* the term ...
	Fig 15.		*S = sleep, f = feeding, c = crying.*
61	Fig. 18.	Fig. *18.*	Fig. *19.*
		(horizontal axis) *F*	*300 mts. after last meal.*
		(vertical axis O^2 per litre/hr.) 4.0, *3.0*, 3.0 ...	4.0, *3.5*, 3.0 . .
	Fig. 19.	Fig. *19.*	Fig. *18.*
		(horizontal axis)	*Time of day in hours.*
62	12	The results are recorded in App. I, *App. II* ...	The results are recorded in App. I ...
63	3	... taken for a least *4*—6 hours. The	... taken for at least *3*—6 hours. The
73	Table 5.	A table summarizing Fig. *21* ...	A table summarizing Fig. *23* ...
80	34	... Only *4* out of 60 cases lie ...	... Only *6* out of 60 cases lie ...
81	15	... the younger not later than *4*—6	... the younger not later than *3*—6
89	4	66.44730 + ...	66.4730 + ...
91	14	as $C = W^b \cdot$ will be ...	as $C = W^b \cdot k$ will be ...
	24	page *76*, viz., that standard ...	page *74*, viz., that standard ...
92	Table 6.	(in column 6: Normal)	
		(German) Schadow *1*	Schadow *41*
		Total: *7*	Total: *47*
		Sum: *99*	Sum: *139*
93	5	... the exponent of $^2/_3$ cannot ...	... the exponent of $^3/_4$ cannot ...
95	28	and 0.*8*12 with 99 per cent ...	and 0.*9*12 with 99 per cent ...
	32	*transformed to a percentage ... fall*	(delete whole line)
96	Table 8.	(in column F) *0.9* (P < 0.01)	*9.2* (P < 0.01)
98	Fig. 27.	(vertical axis) ... 200, *100*, *50.*	... 200, —, *100.*
101	Fig. 30.	Franch materials showing ...	French materials showing ...
107	Table 9.	(in 4. line) $C = W^{2.538} \cdot 0.00832$ ...	... $C = H^{2.538} \cdot 0.00832$ ...
		(in 5. line) ... + 10.7 to — 9.4	... + 10,4 to — 9.4
		(in 9. line) ... $C = W^{2.917} \cdot 0.00189$ ...	... $C = H^{2.917} \cdot 0.00189$ ...
		... + 24.*1* to — 19.9.	... + 24.*9* to — 19.9
109	15—16	... elaborated by Karlberg-Lind *1947*, *1951*).	... elaborated by Karlberg-Lind *1950*).
111	12	seen from Fig. 36. page 12*0*).	seen from Fig. 36, page 12*1*).
	26	... S = "capacitance surfase" ...	... S = "capacitance surface" ...
115	18	$C = 955 \cdot S + 2.17$ (see page 111)	$C = 41.5 \cdot S - 2.17$ (see page 111)
121	1—2	... and *from the residual standard deviation* of the line itself.	... and *the sampling error* of the line itself.
130	Fig. 41.		*(B) from a newborn, using the modification of the method.*
131	Table 12.	(in 2. line) L. S. *8.78* ...	L. S. 2.78 ...
137	20	children exceeding a body weight ...	children *not* exceeding a body weight ...

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INTRODUCTION

During the past fifty years much attention has been paid to the so-called basal metabolism or, more correctly, the minimal energy metabolism under standard conditions, or standard energy metabolism. As far as adults and older children are concerned, such determinations have formed one of the routine methods of clinical examination. This is not the case with infants and although a great many methods have been elaborated for infants, no method has been found serviceable in clinical practice.

Apart from demanding sufficient reliability a method should fulfil the following conditions:

1) It should be simple and easily managed and should not necessitate any prolonged special training. A single person should be able to take charge of it.

2) The necessary apparatus should not be very expensive or costly to use.

3) No complicated calculations should be required.

4) A complete examination should not occupy a long time.

In order to assess the standard metabolism of an individual, one must have normal values, i. e. standard values. No such wholly acceptable values are available for infants.

The purpose of the present work is to supply this need for infants, i. e. children from birth up to one year of age, as follows:

1) By working out at method suitable for routine clinical application for the determination of a standard metabolism.

2) By fixing standard values for this method, based on normal

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material and comparing the results thus obtained with a suitable correlation factor.

During the present investigations, it has been found that owing to the special conditions which arise in newborns it is preferable to distinguish between methods used for infants in the first week of life and later. A special method has been elaborated therefore for the study of the standard metabolism of newborns. In its present form this method is not immediately applicable to practical clinical work.

Chapter 1

EARLIER STUDIES OF INDIRECT CALORIMETRY IN INFANTS[1]

A brief summary

The energy metabolism of an individual, of which the so-called basal or standard metabolism forms a special branch, has been studied chiefly, by *indirect calorimetry*, i e. calculated from a measurement of the gaseous exchance between a living subject and its surrounding atmosphere.

Direct calorimetry, i. e. the measurement of the quantity of heat produced by a subject, on the other hand has been employed only by a small number of scientists. For the latter kind af measurement, a much more extensive technical equipment is necessary. It demands a technical knowledge and is more difficult to perform. The direct calorimetry has been used, mainly to compare results with indirect calorimetry, and also to study the regulation of body temperature. It has not been used exclusively, as a clinical method, for the determination of the so-called basal metabolism of an individual. Accordingly, direct calorimetry has not been used for any analysis in the present work.

The modern science of metabolism was initiated by *Lavoisier* who showed that energy was produced in the body by the process of oxidation. He made the fundamental observation that the amount of oxygen used and carbon dioxide produced depend primarily on food, activity and temperature.

[1] Appendix V has been compiled for the purpose of facilitating a synopsis of the achievements of earlier authors and their followers. Year of publication, type of apparatus, measured quantities, length of observation periods, time of observation in the day or night, food taken before examination (if any), type of calculation and the number and type of children examined, have been recorded with some brief comments.

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In 1849, 1850, *Regnault-Reiset* published a description of the first apparatus used for studies of the gaseous exchange according to the closed system principle. In 1862—1863, *Pettenkofer-Voit's* apparatus appeared employing the open system. Both methods are classics, having served as models for a long series of subsequent apparatuses and are still doing so.

In 1866, *Voit* began to compute, from substances oxidized in the body, the quantity of heat produced from the destruction of those substances. In other words, he introduced a mode of calculation for indirect calorimetry. The followers of *Voit,* e. g. *Rubner, Atwater, Müller, E. Voit, Cremer, Lusk* and *Cathcart,* formed their own conceptions. Rubner was the first to devote attention to determinations in the case of infants.

However, *Forster* (1877) published, before anyone else, investigations regarding gaseous metabolism in infants. At Munich in Germany, he employed an apparatus, after Pettenkofer-Voit's system. He determined the liberation of carbon dioxide during one-hour periods in children including two infants, 14 and 16 days old, respectively.

After a new modification the apparatus was used by *Rubner* in Berlin, and together with *Heubner,* he initiated his famous studies on infants, with a first publication in 1898. These two scientists originated a whole school of research with names such as *von Bergmann, Bardt, Edelstein, Birk, Niemann, Frank* and *Wolf.* Their work continued until 1913. At that time, observations concerning some ten infants had been printed (see App. V). These investigations mainly covered long periods and only the production of carbon dioxide and aqueous vapour were determined.

At the same time apparatuses according to Regnault-Reiset had been employed for the studies of infants The Italian scientists *Mensi* (1894) and *Poppi* (1900) determined both the oxygen consumption and the excretion of carbon dioxide, by the closed circuit system for newborns and atrophic infants respectively. Their methods were not described in any detail.

Scherer (1896) and *Babák* (1902), Czeck scientists, examined newborns using different apparatuses, though both were con-

structed on the Regnault-Reiset's principle. Scherer performed a considerable number of observations, but his method has been criticised by other researchworkers (e.g. Hasselbalch 1904, Benedict Talbot 1914, 1915).

The Germans *Zuntz-Oppenheimer* (1908) devised the first apparatus according to Regnault-Reiset's principle, ~~and it seems to have been~~ constructed thoroughly from a technical point of view.

At Düsseldorf, *Schlossmann-Murchhauser* (1908) carefully tried and checked the suitability of this apparatus with regard to infants. Up to 1914 they performed a series of short-period observations on infants (see App. V). After their death some of their observations on newborns and prematures were published in 1933.

The Dane *Hasselbalch* was among the first to determine both the oxygen consumption and the carbon dioxide production, by using an open circuit. His investigations of newborns, published in 1904, has attracted more and more attention and still remains useful.

In the United States of America, at the beginning of the 20th century, very active groups of researchworkers began to form round Atwater and Lusk. Benedict was particularly notable among them. As far as investigations on metabolism in infants are concerned, *Howland* (1911, 1913) comes first with his still helpful comparisons between direct and indirect calorimetry. In 1911, *Carpenter* and *Murlin* published an investigation of energy metabolism in newborns, defined as the difference between the metabolism of the mother immediately before and after delivery. This method, however, is unavoidably lacking in accuracy.

In 1914, *Benedict-Talbot* issued their first monograph from the Carnegie Institution of Washington. In this, Benedict's apparatus and methods were thoroughly discussed. After three additional monographs on children, a whole series of publications by these authors appeared (see App. V), the last one by *Talbot* in 1938. Newborns, prematures, normal and under-weight children and twins were subjected to observations. *Flemming*, in 1921 and 1924 published observations on under-weight children and applied approximately the same methods.

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Murlin (1915) elaborated a method that in some respects differs from that of Benedict-Talbot. He and his collaborators (up to 1925), performed a rather extensive series of observations, particularly on prematures and newborns.

The newborn stage was studied also by *Marine*, the pathophysiologist (1922), a special apparatus being used.

About that time, i. e. in the twenties, the interest of German scientists in metabolism was again renewed. *Kestner* and *Knipping* seem to have come particularly to the fore. Knipping constructed a number of apparatuses for the study of different aspects of metabolism, all according to Regnault-Reiset's principle, including observations on infants. The apparatuses are carefully described, but no reports are given of any independent results. Kestner-Knipping reported, however, standard values for different ages, including infants (1924). Knipping's apparatus was later adopted by *Baer* for observations on infants (1929), and by *Schadow* (1930, 1932, *Beck* (1931), *Essig* (1932) and *Mordhorst* (1933) (see App. V). After that time, no German publications in this field have been traceable in the literature.

Also in France, with her old traditions of study in the sphere of metabolism, determinations by indirect calorimetry on infants started towards the end of the twenties. Publications by *Janet-Bochet* appeared in 1929, 1931 and 1933, and by *Garot*, in 1933. All these investigations were performed by open circuit apparatus. Before that, *Weiss* (1908) and *Fouet* (1924, 1925) had carried out investigations on infants by methods similar to those used by *Scharling* in 1840 for observations on animals. The entire apparatus consisted of a small, completely closed respiration chamber without any circulation. From this, samples were taken after a few minutes for gas analysis. The method cannot have producted reliable results. This is obvicus from Fouet's values, compared with those obtained by other French authors. In 1934 *Lesné-Nattan-Larrier* published exsperiences with a closed system method, which had been intro duced by *Plantefol* in the previous year.

Thus in Europe there was very little metabolic study to record.

In the United States, on the other hand, the metabolic research in infants, from the days of Benedict-Talbot and Murlin and others, was revived by *Levine* and his co-workers. Since 1925 they have published a series of investigations. Their studies comprise normal and under-weight infants, as well as prematures, with regard to their so-called basal metabolism and also their daily energy requirements. They have set forth standard values, analysing the influence of different foods on metabolism, etc. Up to 1936 they employed a modified Benedict apparatus, and then they adopted an open system method.

The Americans *Clagett-Hathaway* (1941) and *Benjamin-Weech* (1943) have performed measurements using the open system on eight and four infants respectively. A few measurements extended over a long period of time.

In Scandinavia, the metabolism in infants has attracted a certain amount of attention in recent years. The Finns *Räihä* (1936) and *Malm* (1948, 1951) and the *Swede* Kaijser (1944) have given accounts of different methods suitable for infants, but the observations thus recorded are few.

The fundamentals of the method used in this work has been previously outlined in 1948 by *Karlberg-Lind*, and in 1948, 1950, 1951 by *Karlberg*, but since then has been modified in sereval respects.

Apparatus used for indirect calorimetry

The apparatuses described in the literature can be divided into two main groups, viz., those conceived according to the principle of the open system or to that of the closed system. Both have developed approximately simultaneously.

In the *open system*, the child is breathing in a respiration chamber through which atmospheric air is flowing. The reduced amount of oxygen in the outgoing air and the increased amount of carbon dioxide and, sometimes the aqueous vapour are determined by a time unit. In the case of the *closed system*, the subject is breathing in a respiration chamber which forms part of a closed system in which the gas circulates.

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The carbon dioxide and possibly the water vapour, which the child discharges, is absorbed, and the oxygen consumed is measured, either as a decrease in the volume of the system or as the amount of oxygen that must be added in order to keep the volume of the system constant. In this connection, attention must be paid to such variations as may occur in pressure, temperature and concentration of carbon dioxide and aqueous vapour in the system. After the examination, the amount of carbon dioxide absorbed and aqueous vapour produced can be assessed also for certain time periods.

These two fundamental principles for indirect calorimetry are often named after the scientists who have introduced them, i. e. *Pettenkofer-Voit* and *Regnault-Reiset* for the open and closed system respectively.

Pettenkofer-Voit. The first apparatus used for metabolic observations on infants (Forster 1872) was that of Pettenkofer-Voit, as modified by *Liebermeister* (1870). After *Wolpers'* modification (1896) the apparatus was adopted for use by Rubner and Heubner and their followers. As years went by, only minor changes were introduced. Only the production of carbon dioxide and of aqueous vapour were measured.

This was done gravimetrically after absorption for some fixed time, usually several hours. For this reason, only long-time experiments were undertaken for the most part, covering several 48-hour periods, with short intervals for the administration of food. Accordingly, no determinations of the so-called basal metabolism took place. After 1913, no work based on this method was published.

The apparatus must have been big and unwieldy. Its reliability cannot have been particularly good, in spite of the long observation periods. Apart from that, the oxygen consumption could not be estimated.

Hasselbalch. In the thirties, apparatuses using an open system were again brought into use, owing, no doubt, to the fact that at that time it was generally known how to execute reliable gas analyses. Hasselbalch's method, published in 1904, constitutes a brilliant exception.

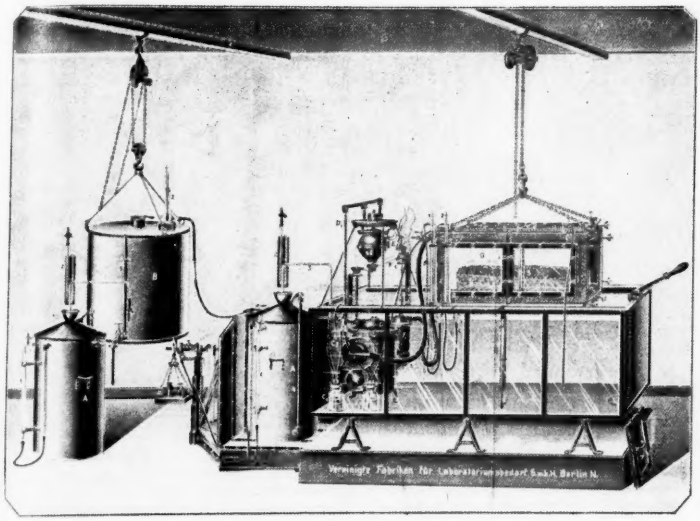

Fig. 1. *Zuntz-Oppenheimer's apparatus. (Biochem. Ztschr., 14: 361, 1908.)*

His apparatus, which is characteristic of the open system, can be said to be a model for the apparatuses of recent times, where the ingoing air volume is measured in a gasometer, while the oxygen and carbon dioxide concentractions in the outgoing air are being analysed. For these analyses Hasselbalch used *Pettersson's* apparatus which is stated to have produced results of such reliability that Hasselbalck felt justified in concluding that metabolism can be determined with accuracy to within 2 per cent of the actual value, and the respiratory quotient to within ± 0.002.

Zuntz-Oppenheimer. The first apparatus designed for infants, based on *Regnault-Reiset's* principle, which reflects modern technical progress and seems to be of some value in practice is that of Zuntz-Oppenheimer (1908).

The construction will be seen from Fig. 1. The child is placed in a respiration chamber with glass walls. It is immersed in a big water basin in which an absorption vessel and a pump are also placed. By these means, the air ~~density~~ Volume of the system is kept under satisfactory control. The oxygen consumption of the child is measured by the addition of oxygen up to a constant volume, after the absorption of the carbon dioxide with potassium hydroxide, from which it was then released for measuring. Schlossmann and

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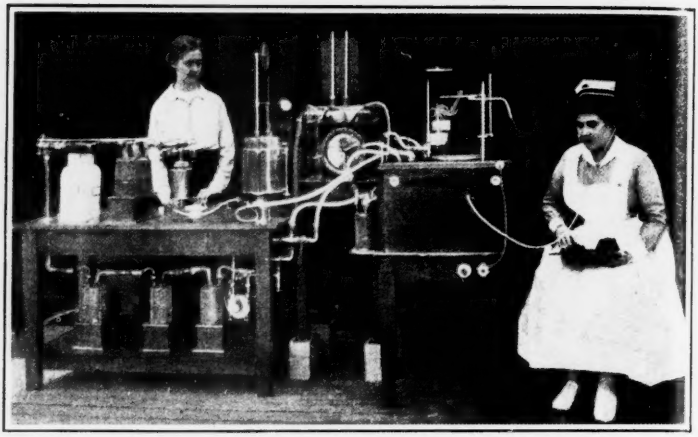


Infant respiration apparatus as used at the Directory for Wet-Nurses.

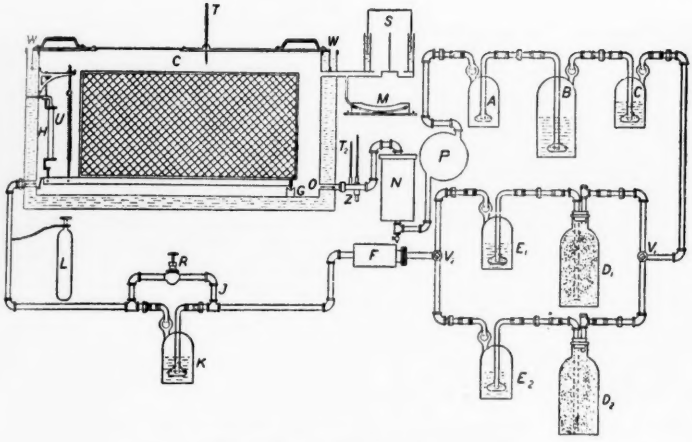
At the right are the respiration chamber, the tambour, and kymograph for recording the muscular activity, also nurse counting pulse-rate. At the left is the absorbing system, with the carbon-dioxide absorbers and spirometer on the upper shelf of the table, the water-absorbers and blower on the lower shelf. The gas-meter for measuring oxygen may be seen in center.

Fig. 2. *Benedict-Talbot's Apparatus. (Benedict, G. B. & Talbot, F. B., Metabolism and growth from birth to puberty. Publication 302, Carnegie institution of Washington, 1921, p. 30.)*

Murschhauser carefully checked the method, inter alia by alcoholic tests, obtaining mean errors for the carbon dioxide of + 0.42 per cent, and for the oxygen of — 2.00 per cent. In order to secure such a degree of accuracy, the air in the respiration chamber must be analysed for carbon dioxide both at the beginning and at the end of each test. The calculations for an alcohol test cover more than a large page.

The method is certainly exact but, at the same time, is very laborious. It is evident from Fig. 1, that it was necessary to fix pulleys to the ceiling in order to lift the solid respiration chamber from the water bath. Schlossmann and Murschhauser were alone in adopting this apparatus.

Benedict-Talbot. The first apparatus to be used widely in investigations on infants employing the closed system principle was that designed by Benedict. This apparatus was used by Benedict and Talbot for their extensive investigation series. Since it has



Detailed scheme of respiration apparatus.

C, chamber; *W*, *W*, water jacket; *O*, outgoing air-pipe; *Z*, psychrometer; *N*, muffler; *P*, blower; *A*, acid trap; *B* and *C*, Williams water-absorbers; V_1 and V_2, 2-way valves; D_1 and D_2, carbon-dioxide absorbers; E_1 and E_2, air-dryers; *F*, sodium bicarbonate can; *J*, by-pass; *R*, valve; *K*, air moistener; *L*, oxygen cylinder; *I*, ingoing air-pipe; *S*, spirometer; T_1 and T_2, thermometers; *M*, manometer; *U*, spiral spring; *H*, pneumograph.

Fig. 3. *Benedict-Talbot's apparatus shown diagramatically. (Benedict, G. B. & Talbot, F. B., The gasceous metabolism of infants with special reference to its relation to pulse-rate and muscular activity. Publication 201, Carnegie institution of Washington, 1914, p. 35.)*

been the model for a number of later methods, it will be dealt with more fully below. (It was most extensively described in Benedict-Talbot's first monograph in 1914, but a good account is given also in Abderhalden's Handbuch der Biologischen Arbeitsmethoden, Abt IV, Teil 10, 1926). Its construction and principle will be seen from Figs. 2 and 3.

The child is placed in a respiration chamber, built as a rectangular box, with a window lid, closed hermetically by means of a water-trap. The chamber is hermetically sealed except for outlet and intake tubes. In the respiratoin chamber, the child consumes oxygen and discharges carbon dioxide and acqueous vapour. The air in the chamber is thus changed and pumped from the chamber into a system of flasks that will absorb the moisture and the carbon dioxide. The air is again moistened and forced back into the respiration chamber. But before reaching the chamber oxygen ~~back~~ added to the returning air, to make up its oxygen deficit, and keep the volume

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of the system constant. This administration of oxygen takes place intermittently. For this reason, a spirometer is coupled to the system, in order to provide for any expansion or contraction of the air.

The oxygen consumption is obtained from the administered amount of oxygen, and is corrected for any variations that may be noted in the volume of the system at the beginning and at the end of an observation period, and this reading is taken from the spirometer at a constant pressure (which is controlled on a sensitive manometer). This amount of oxygen is corrected for barometric pressure, temperature and moisture. The latter value is registered by a psychrometer coupled to the system. Variations in these three quantities, if any, during the observation time have to be included in the calculations. Since the oxygen determination is based on a measurement of the volume, *the system must be maintained at a constant temperature in addition to being absolutely air-tight.* For that reason, the respiration chamber is provided with a water-jacket i. e. double walls and a double bottom, the intervening space being filled with water. The temperature of the water is kept constant either by heating with a burner placed below the chamber, or by cooling by means of pieces of ice placed in the water, in the cold or hot seasons respectively.

The *discharge of carbon dioxide* is obtained from the increase in weight of the absorption flasks during the time of observation. Before each observation period, the absorption system must be replaced by a new one.

The *movements of the child,* if any, are registered by peumography on a sooted pneumograph paper. The *pulse rate* is obtained by auscultation by means of a stethoscope. In order to quieten the child and thus to secure reasonably constant physical conditions in the system about 30 minutes are require for *he preliminary period.* The *observation time* occupies 20—30 minutes for young infants and about 15 minutes for the older infants. The duration varies with the length of time during which a child keeps calm. Frequently, the observation periods have to be repeated again and again.

The determinations of the volume of the system and consequently, the oxygen determinations were extremely susceptible to slight movements by the child, because of the fact that the concentrations of the carbon dioxide, oxygen, humidity and temperature, would rapidly change in the respiration chamber. Therefore, the production of *carbon dioxide was used for the metabolic calculation* in each separate observation period, while the oxygen consumption was applied only for the assessment of the average respiratory quotient for several subsequent observation periods on the same child.

Apparently this method is fairly reliable, as will be seen from the accounts of the alcohol tests, the error is restricted to ± 2 per cent for the values for carbon dioxide and oxygen.

Murlin. Murlin's apparatus, first published in 1915, but best

described in Abt's Pediatrics in 1923, is closely related to Benedict-Talbot's, though differing in certain aspects.

The absorption system consists of Benedict's universal apparatus, but the oxygen administration has been made automatic. Contrary to Benedict and Talbot, Murlin was of the opinion that the *caloric value* should be calculated *from the oxygen consumption* and not from the excretion of carbon dioxide. Accordingly, the *accuracy in oxygen determinations had to be increased.* This was achieved by keeping the temperature in the system more constant, by means of an air thermostat built in the form of a respiration chamber, and by analyses of carbon dioxide and humidity at the commencement and end of each observation period. The movements of the child were recorded from an air bed on a kymograph, the pulse registration took place automatically from a cuff applied to the thigh. In some of the tests the respiration was recorded.

Extremely careful alcohol tests are reported in detail. In spite of all this care, the errors sometimes amounted to 5 per cent owing to the difficulty in maintaining a constant temperature.

Levine. In 1926—27, Levine, and his co-workers, presented some further improvements in the method, by making the *temperature in the respiration chamber still more constant.*

The chamber was partially immersed in approximately 1,000 litres of water at a temperature of 15° to 16° C. During the preliminaries, when the child was asleep, the temperature of the water-jacket was carefully adjusted to the heat production of the subject in order to keep the temperature of the chamber uniform. In individual experiments varying from 2 to 6 hours, the fluctuations never exceeded 0.5° C., and very often stayed below 0.2° C. The chamber is ventilated by the Benedict universal respiration apparatus. The absorption system has been somewhat modified to obtain more accuracy. The *moisture in the system* is measured by means of a hair hygrometer and by regulating the rapidity of the gas flow it is restored at the end of each investigation period to the rate it was at the beginning. The oxygen is admitted by the manipulation of a small air-tight cylinder with a regulator of such delicacy as to keep the volume practically unchanged during the whole time. This cylinder is weighed before and after each investigation period, with a precision within 10 mg. The activity and pulse of the child are registered visually every 10—15 minutes.

Levine and his co-workers used this closed system up to 1940, when it was replaced by an open system apparatus. During the years 1925—40 alcohol tests were regularly made, showing a mean error of about — 3.6 per cent for oxygen and about —

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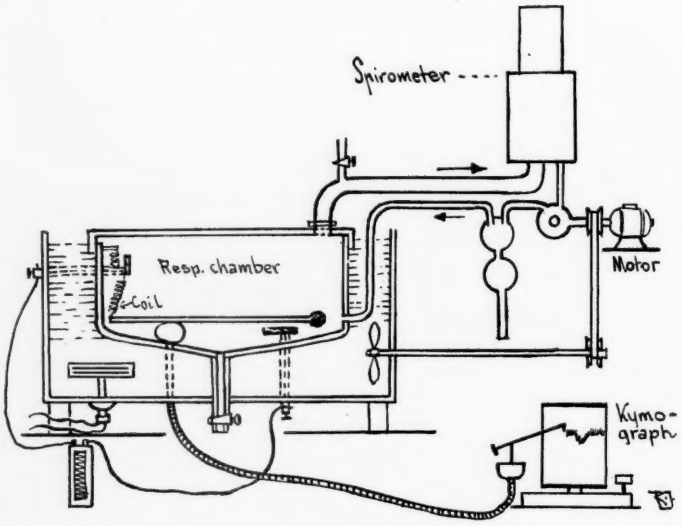

Fig. 4. *Knipping's apparatus shown diagramatically.*

2.2 per cent for carbon dioxide, with maximal differences in both instances of — 4.5 per cent. Judging from these tests and duplicate determinations on infants, Levine assesses the probable experimental error of the method to be less than ± 5 per cent.

Knipping. Another modification of the closed system is to be noted. It is of German origin and has been used extensively in the country of its originator, viz., Knipping's. The first publication appeared in 1924. It was followed by several. The best descriptions of his apparatus for infants are to be found in the work he published jointly with *Rona* (1928), and in the handbooks on "Gasstoffwechsel Apparat" issued by Dargatz at Hamburg (obviously *Knipping's* technical collaborator). The apparatus differs in some of its fundamental aspects from that of Benedict. The construction will be seen from Fig. 4.

Like the American apparatuses the respiration chamber is equipped with a water-jacket a top lid spirometer, a circulation pump, absorption devices for carbon dioxide and water vapours and a pneumo-registration method for recording the movements of the child. The pulse rate is recorded

by the stethoscope. In order to keep the temperature as even as possible, the water-bath surrounding the respiration chamber is electrically heated. The temperature is regulated by means of a thermocouple in the chamber and an electric stirrer. *The oxygen and carbon dioxide determinations are, on the other hand, volumetric.* The discharged amount of carbon dioxide is absorbed in a water-cooled flask with potassium hydroxide. The oxygen consumption is obtained by means of a *continuous graphic registration on the spirometer from decreases in the volume of the system.* As a matter of course, this value for oxygen consumption must be corrected afterwards for barometric pressure and temperature (mean value from four thermometers), as well as for the humidity in the system which is read from a hygrometer. In addition, correction is made for such amounts of carbon dioxide as remain in the system at the end of each observation period, as calculated from a carbon dioxide analysis of the air in the system and determinations of its volume. The amount of carbon dioxide, absorbed in the potassium hydroxide flask during the observation period, is measured volumentrically at the end of the test. This is done by taking out the child. The system is again closed. When an equilibrium is reached, the carbon dioxide is liberated from the potassium hydroxide by addition of sulphuric acid. The amount of carbon dioxide will equal the increase in volume after the restored balance in the system (the heat emitted on the administration of the acid must be taken into account). Also, correction must be made for physical release of carbon dioxide. This method likewise requires preliminaries of about 30 minutes to attain an equilibrium in the system, before the actual observations commence. An exhaustive account is set forth of the way of performing an alcohol test. Attempts to trace any results from the apparatus for infants in the literature however, have proved in vain.

The study of the method leaves an impression that it has been carefully planned, except for the volumetric determination of the carbon dioxide. Knipping has published standard values for infants, without, however, producing any original values. Those researchworkers who have adopted Knipping's apparatus (see App. V) have all expressed great satisfaction, except *Mordhorst* (1933) who adds the following criticism: "Die Kohlensäure habe ich in allen Fällen bestimmt, lege jedoch auf die Ergebnisse keine Werht, da sie im allgemeinen zu ungenau sind und daher keine exakten Vergleiche zulassen." Mordhorst stresses the importance of a preliminary period of observation for 30 minutes before beginning the actual observations. As far as can be seen from the literature, Knipping's apparatus has only been employed in Germany.

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During the past decade, there has been a general tendency to return to apparatuses using *the open system* principle, no doubt owing to the fact that, in using this system, it is not necessary to have an absolutely constant temperature in the system. Even during the interval between the Rubner-Heubner period and that of Hasselbalch at the beginning of this century various works based on the open system were published.

Marine. In 1922, Marine brought out a method for newborns (earlier employed by him for smaller animals).

Dry air is sucked up through a simple respiration chamber (I), a water-absorbing vessel (II), a carbon dioxide absorbing vessel (III), a gasometer (and pump). The carbon dioxide production during a fixed observation period is obtained from the increase in weight of (III). The oxygen consumption is computed from the increase in weight of (I + child + II) + the increase in weight of (III). Resulting respiratory quotients are high (in the few original determinations that are recorded). No alcohol tests have been reported.

The method seems very simple but can hardly claim to be reliable.

Janet-Bochet. In 1929, the first reports appeared from the French scientists Janet-Bochet regarding an open method for infants. It was modified and tested during subsequent years.

The child is placed in a rubber silken bag with an opening for the head. A bell-jar fits over the head into the collar of the bag. Inlet and outlet tubes, provided with safety valves, allow the air to pass in and out. A small pump forces air into a gasometer for a standard time interval (mostly 4 minutes). By means of an apparatus for gas analysis, according to *Plantefol,* the air is analysed for its carbon dioxide and oxygen contents. Corrections are made for temperature and barometric pressure. The reliability of the apparatus has apparently been subjected to a careful control.

Alcohol tests have been performed. The mean value of respiratory quotients equals 0.672 (no. = 13), with a range, however, of 0.30—0.90 (next 0.61—0.80).

Delaunois-Verhoestrate deviced in 1942 an open method where the differences in oxygen and carbon dioxidconcentrations between the ingoing and the outgoing air are continuously registered.

The American open circuit apparatus. The majority of investigations on infants, performed in America during the past decade, have been made by *Levine* and his co-workers *Clagett-Hathaway* (1941) and *Benjamin-Weech* (1943) and all used the open system according to the technique defined by *Higgins* and his co-workers (1930, 1939). In addition, Levine and his co-workers have had recourse to *Newburgh's* publications on adults (1937) and Benjamin-Weech to *Lewis* and co-workers' investigations regarding older children (1937). Only certain minor modifications distinguish the one from the other.

Broadly speaking, the course of the investigations is as follows: Fresh air, coming either directly from without or from the conditioned room, is sucked in through a respiration chamber (somewhat varying in size) in which the child is placed. To begin with, the air is allowed to pass straight through the chamber. When the child has been calmed, and a certain gaseous equilibrium is reached in the chamber, the gas mixture escaping from the chamber is trapped in a spirometer, during 3—5 periods of 1—2 minutes each. Time and volume are carefully measured. Gas samples are taken from the accumulated amount for *analysis for oxygen* and *carbon dioxide*, according to the *Carpenter-Haldane* technique. As a rule, duplicate determinations are made for each period. The spirometer volume is corrected for barometric pressure and temperature.

A rising concentration of carbon dioxide during the investigation periods signifies a simultaneous accumulation of carbon dioxide in the respiration chamber. Accordingly, a correction has to be made for this, according to *Higgins-MacLaren* (1939). As regards the moisture in the circulating air, *Levine* and co-workers dry the air before it enters the respiration chamber and, again, before its entering the spirometer. *Benjamin-Weech* do not remove the moisture and they obtain a 100 per cent humidity in the respiration chamber. The rate of circulation is so high as to keep the carbon dioxide concentration at about 0.5 per cent. It must not exceed 1 per cent. The reliability of all these methods has been checked either by repeated analyses of the fresh air or through alcohol tests (see Table 1).

Apparatuses for determinations on infants have been devised in Scandinavia during the past few years.

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Table 1. *A table showing the results of air analyses and alcohol tests from American open circuit apparatuses*

Author	Air analysis A - a Alcohol test A - t	O_2	CO_2	RQ
Gordon et al. (1940)	A - a	20.935 ± 0.006 vol. %	0 ± 0.003 vol. %	
	A - t Mean	— 3.7 %	— 4.5 %	0.662
	Range	+ 0.2 to — 7.7 %	— 0.2 to — 7.3 %	0.669 to 0.650
Dann et al. (1942)	A - t Mean	— 3.6 %	— 4.0 %	0.662
	Range	— 1.8 to 5.5 %	— 1.9 to — 5.2 %	0.667 to 0.657
Clagett-Hathaway (1941)	A - t Mean		+ 0.91 %	0.665
	Range		+ 3.95 to — 2.39 %	0.655 to 0.675
Benjamin-Weech (1943)	A - a	20.939 ± 0.0229 vol. %	0.036 ± 0.0058 vol. %	
	A - t Mean			0.665 ± 0.0137

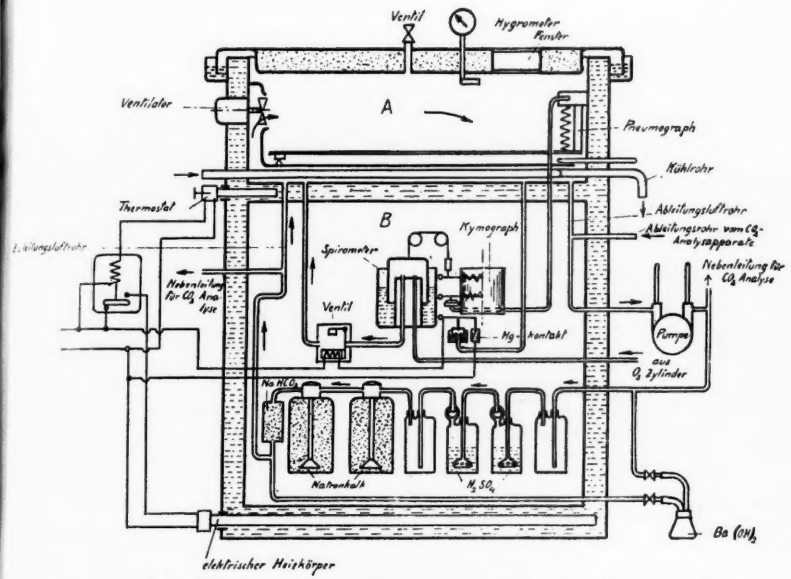

Fig. 5. *Räihä's apparatus shown diagramatically. (Acta Paediat., 18: 453, 1936.)*

Räihä. This method, published in 1936, measures the oxygen consumption according to the principle of the closed system. Great care has been paid to achieve constancy in temperature (see Fig. 5).

Inter alia, the air returning to the chamber is cooled. The respiration chamber is equipped with a fan. The maintenance of the oxygen administration at a constant volume has been made entirely automatic and extremely delicate. The *carbon dioxide* excretion in the circulating system is measured according to the *open principle*. In a shunt, past the carbon dioxide absorption flask, analysis of the carbon dioxide is performed, according to *Kauko*, while the rate of circulation in the system is measured by the passage of gas through a gasometer.

Errors in an alcohol tests amounted to 4 per cent for oxygen and 3 per cent for carbon dioxide.

Kaijser's method (1944) is closely related to this method. In this instance, the administration of the oxygen is, however, arranged more simply, though less carefully. The carbon dioxide concentration is analysed by titration. No alcohol test is reported.

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Malm. In 1948, Malm demonstrated a method for prematures on the same principle as the *Douglas bag*. For the purpose of studying the gaseous exchange during short periods, he constructed a rubber mask combined with a valve and a gas-collecting bag. At the end of the investigations, analyses were made according to Haldane of carbon dioxide and oxygen in samples from the bag. The volume of the bag was determined by means of a gasometer. Many of the prematures endured the mask fairly easily and sleept well.

In 1951, Malm gave some preliminary reports on an open circuit method, where the oxygen concentration was determined continuously with an electrical analyser (Malm-Vuorelainen, 1950).

Conclusion. Apparatuses using the principle of the *closed system* were from the outset simple and less precise. In the course of years they have become more complicated and technically developed, and at the same time more expensive. The object has been to eliminate variations in temperature and gas concentrations in the circulating system. They are far from easy to manage.

Apparatuses using the *open system* principle were at the beginning very big and difficult to handle (necessitated a full time mechanic). Thanks to the availability of satisfactory and exact methods for gas analysis, efficient pumps holding a constant speed and reliable gasometers and owing to the fact that it is possible in this case to manage with respiration chambers of a simpler construction, open system apparatuses have experienced a revival. However, a definite disadvantage remains. The necessary gas analyses requires a well-fitting apparatus and a well-trained staff. Also, they require plenty of time. This is particularly unwelcome as several analyses are needed as a rule for each determination.

From a *practical clinical point of view*, no apparatus appears, in fact, to be well adapted for use.

Chapter 2

AUTHOR'S METHOD

Apparatus

This method was based on *Krogh's spirometer* (1922) in order that an apparatus could be devised suitable for the big hospitals in Sweden. Krogh's apparatus is used in Sweden, and its working principles and the art of managing it are familiar to all. It is built on the principle of the *closed system* in which the carbon dioxide is absorbed by soda lime and the container for the soda lime is built into the spirometer. Only *the oxygen consumption* is measured and this value is obtained from the decrease in the volume of the system, which is recorded continuously on a kymograph.

In addition to the Krogh's spirometer, a respiration chamber and a circulation pump were needed for the investigations on infants.

The respiration chamber (see Fig. 6) was built in two parts. It consists of: (1) a bottom plate made of a folded and soldered metal sheet, with a 15 mm thick heat-isolating cork plate for the child. Round the edge runs a 50 mm high and 25 mm broad trap for water. At the foot of the chamber there is a semicircular perforated tube for the incoming air and at the head there are two tubes for the outgoing air which join one another. (2) A plastic cover fits into the water-trap and rests on the bottom plate.

Two respiration chambers, of the same construction, but differing in size, have been used. At first a smaller one holding

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about 30 litres was used, but it was soon replaced by the larger chamber with a capacity of about 42 litres. This chamber, called the *standard chamber*, has been found to be the most suitable for the determination of standard metabolism. The measurements of the chambers and a comparison with earlier apparatuses will be seen in Table 2.

Table 2 *The size of the respiratory chamber in different apparatuses.*

Apparatus according to	Respiratory chamber				
	length cm.	breadth cm.	height cm.	volume lits.	
Hasselbalch (1904)				15	Open system.
Weiss (1908)				60	
Bahrdt (1910)	122	100	80	1,820	Open system.
Edelstein (1910)					
Grafe (1911)	130	100	80	1,040	Open system.
Niemann (1913)				150	Open system.
Benedict (1914)	77	37	25	71	
Talbot (1914)					
Murlin (1915)	76	32	30	73	
Marine (1922)	56	25.5[1])	25.5[1])	23.5	For newborns. [1]) Cylinder
Fouet (1924)				50—60	
Levine (1927)				120	
Knipping (1928)	80	30	40	96	
Räihä (1936)	92	39	41	147	
Levine (1940)				21	Open system. For prematures
Benjamin-Weech (1943)	101	45.7	35	161	
Kaijser (1944)	110	50	40	220	
Author's:					
Standard cover	72.5	29.5[2])	23[2])	42	[2]) Approx. semi-cylindrical.
Small cover	49	27[2])	24[2])	30	

The circulation pump is in a bellow-like form and is worked by a small electric motor (see Fig. 6). Its capacity can be easily varied, either by changing the rapidity of the motor or through changing the range of the bellows. Its maximal capacity amounts to 18 litres per minute. This type of pump offers two distinct advantages: (1) With the absence of any rotating axis in the system, the pump can be relied on to be air-tight. (2) By

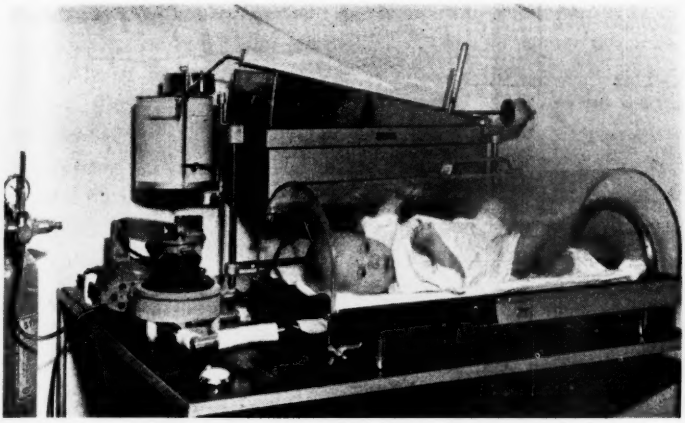

Fig. 6. *Author's apparatus.*

means of the spirometer, the rhythmic movements of the pump are transferred to the recorder of the kymograph, and is kept moving continuously up and down, while the paper moves gradually sidewards and the recorder itself slowly descends. Had the recorder not maintained these rhythmic movements, the friction against the paper would have been much more marked and the recorder would have stuck, and thus resulted in an uneven curve. The peaks of each pump stroke form a straight line with a constant fall of the spirometer.

The Krogh's spirometer employed is the standard form produced by A/B Kifa for several years in Sweden.[1] There are some modifications viz. the writing apparatus has been altered in order to make the recorder run vertically with the spirometric filling changes (see Fig. 6). Finely granular soda lime is used instead of the coarse standard type. A somewhat thicker kymograph paper is necessary in order to withstand the frequent movements of the recorder. The temperature readings in the

[1] All additions to the Standard Krogh's spirometer are available from A/B Kifa, tSockholm, Sweden.

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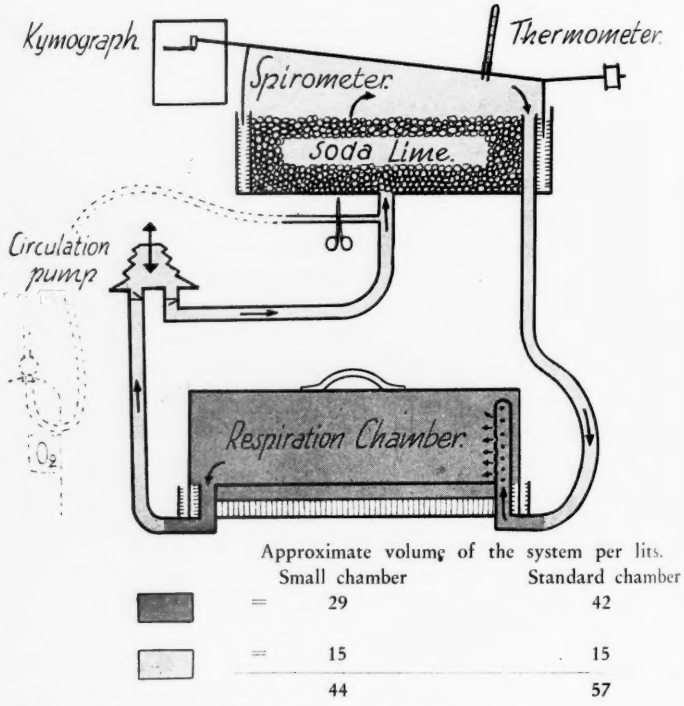

Fig. 7. *Author's apparatus shown diagramatically.*

system are taken in one place,[1] viz. in the spirometer. For this purpose the standard thermometer of the spirometer is employed. The temperature is read to within 0.1°—0.2° C. At the beginning of each investigation, oxygen is administered from an oxygen cylinder via a rubber tube to the spirometer. There is no special device for recording the *child's activity*. As will be shown later, this activity will appear automatically on the kymograph curve. The moisture is not measured.[1] The time of the test is recorded by a stop-watch, and the atmospheric pressure by an ordinary laboratory barometer. The child lies on a small blanket and linen sheet which is placed over the cork plate.

[1] During a usual determination.

Connection. The three large parts of the apparatus viz. the spirometer, the respiration chamber and the pump are connected by means of rubber tubes having an interior diameter of 20 mm. This is illustrated in the diagram in Fig. 7, and can be seen in the photograph of the apparatus in Fig. 6. The *total volume of the system* with the standard respiration chamber varies between 50 and 58 litres, according to the size of the child and the degree of filling of the spirometer.

Circulation. The circulation of the system runs as follows: Starting in the respiration chamber, where the child produces carbon dioxide, moisture and heat, and at the same time consuming oxygen, the air passes via the pump to the bottom of the spirometer through the carbon dioxide filter and enters the spirometer and from there returns to the foot of the respiration chamber. It is important to have the pump coupled between the head of the respiration chamber and the carbon dioxide filter in the spirometer, because the biggest obstacle to the circulation of the air is to be found in the carbon dioxide filter. If either of the two water-traps, i. e. the spirometer and respiration chamber water-traps, were situated in between the pump and the soda lime, then it would be impossible to avoid the strong variations in pressure and big oscillations in the water trap after every pump stroke. It is to be noted that the gases circulate from the head of the respiration chamber to the soda lime container (see diagram Fig. 7), and thus the air contains no carbon dioxide when it enters the spirometer, and the expired air of the child leaves the respiration chamber as early as possible. Both these factors hasten the establishment of a gaseous equilibrium in the system.

Calibration of the spirometer. Before applying a spirometer to determinations on infants, its calibration factor should be controlled, i.e. a fall of 21 mm. is equivalent to one litre which is the most usual arrangement for a Krogh spirometer. In the present work the calibration is performed as follows:

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The soda lime container is removed and the spirometer replaced. The ingoing and outgoing tubes of the spirometer are connected by a rubber tube, and the tube for the administration of oxygen is detached from the reduction valve of the oxygen cylinder and is provided with a medium-sized glass funnel. By means of a 500 ml. dilution bottle, the spirometer is gradually filled with water at the exact room temperature. The gasometer then rises step by step. Each step, corresponding to 500 ml. of water, is marked on the stationary kymograph paper and equals an increase of 500 ml. in the spirometric gas volume. When the spirometer is filled with as much water as to bring the gasometer up to the highest point, the calibration factor of the spirometer is calculated. In this connection, the equality of the calibration factor in different positions of the spirometer is checked.

One or two calibrations are performed and they need not be repeated unless the spirometer is subjected to rough handling.

Tests for tightness. Tests for tightness must be made at intervals. The easiest way of doing this, is to fill the respiration chamber and the spirometer with room air. This can be done most simply by raising the gasometer before the system is closed. It would be incorrect to use oxygen for this purpose, because as soon as it issues from the oxygen cylinder the pressure falls rapidly and the gas cools. The pump is brought into action, and the kymograph is made to record the volume of the system for 20 minutes and this is equivalent to a complete circuit of the kymograph drum. In the case of any leakage the volume usually increases, because the pressure becomes negative with each pump stroke. The tightness of the system can be checked by placing a small load on the roof of the spirometer. The author uses an O_2 cylinder spanner for this purpose. Any leakage will then cause a reduction in the volume of the system.

Investigation procedure

A steel spring keeps the kymograph paper in position and this is covered with a tape 10 mm. broad. The purpose of this is to prevent the recorder from being caught by the spring, as the kymograph will describe several circuits during the course of an investigation. The child is laid on the bottom of the respiration chamber and is enclosed with the plastic cover (see Fig. 6).

One of the coarse rubber tubes is detached from the spirometer. As soon as the plastic cover for the respiration chamber is immersed in the water-trap, the spirometer is completely emptied by applying slight pressure on the spirometer. The rubber tube is reattached and the system is entirely closed. The *spirometer is filled to its maximal capacity with pure oxygen* from the cylinder. For safety's sake a clamp is fixed to this tube. A basic line is drawn rapidly on the kymograph paper in this maximal position by rotating the drum of the kymograph manually. The pump and stop-watch are started. The pump is adjusted to a certain frequency and capacity viz., 10—12 lits./mt. for young infants, 14—16 lits./mt. for more advanced ones, and the investigation commences.

At the end of each *5 minute period* the pump is stopped for a few seconds, i. e. long enough to obtain a distinct notch in the curve. In the course of the investigation, *observations on the activity of the child* are recorded by a simple code system (see Fig. 8). Every 5 minute period is defined and it then forms the basis for the subsequent calculation (see below). The record of the activity of the subject will be checked by examining the appearance of the kymograph curve, because the curve becomes uneven as soon as the subject moves (see Fig. 9). As regards the length of an investigation, 5—15 minutes will elapse as a rule before a child is quiet. After that another 10—15 minutes are required to achieve a balance between gas and temperature in the system. Then the investigation will continue for about 30 minutes, i. e. allowing a suitable space of the time for the calculation of the oxygen consumption (see below). *An investigation will thus take about 60 minutes to complete.* The child is taken out of the chamber and the kymograph paper is removed. For a typical curve see Fig. 8. During the investigation, the temperature is taken every fifteen minutes with the thermometer on the spirometer and the value is recorded on the aforementioned record. At the conclusion of an investigation the barometric pressure is taken.

The volume record. The position of the spirometer at the end of every 5 minute period is recorded in the following way:

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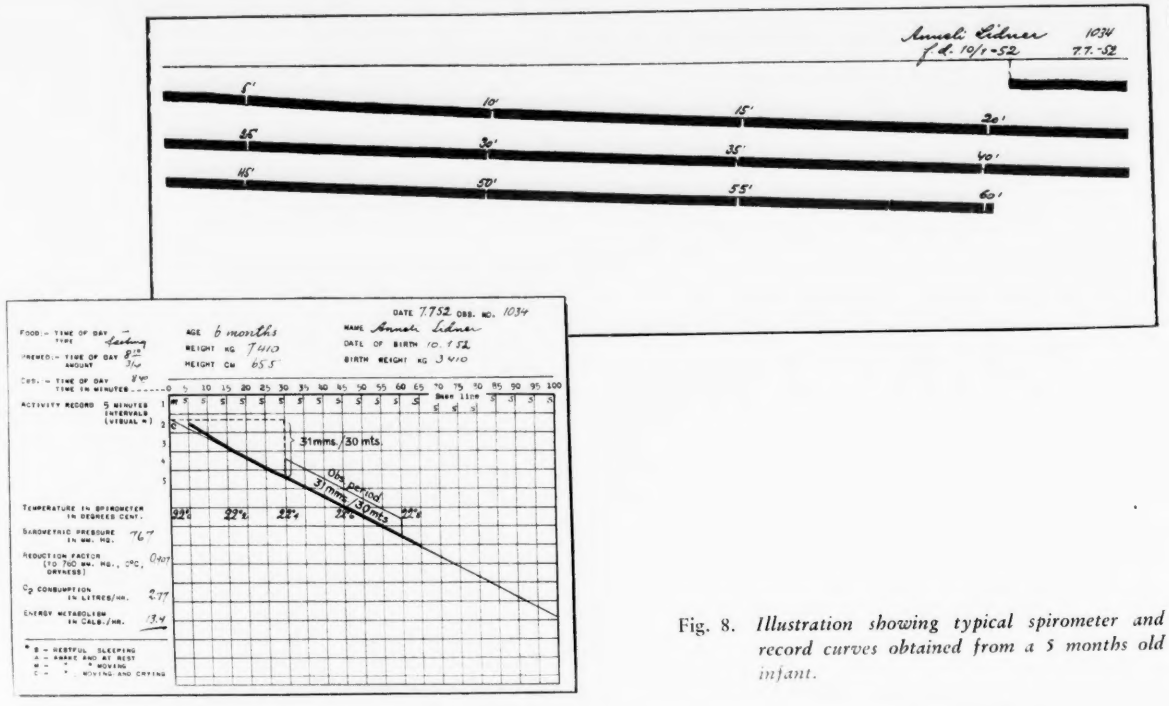

Fig. 8. *Illustration showing typical spirometer and record curves obtained from a 5 months old infant.*

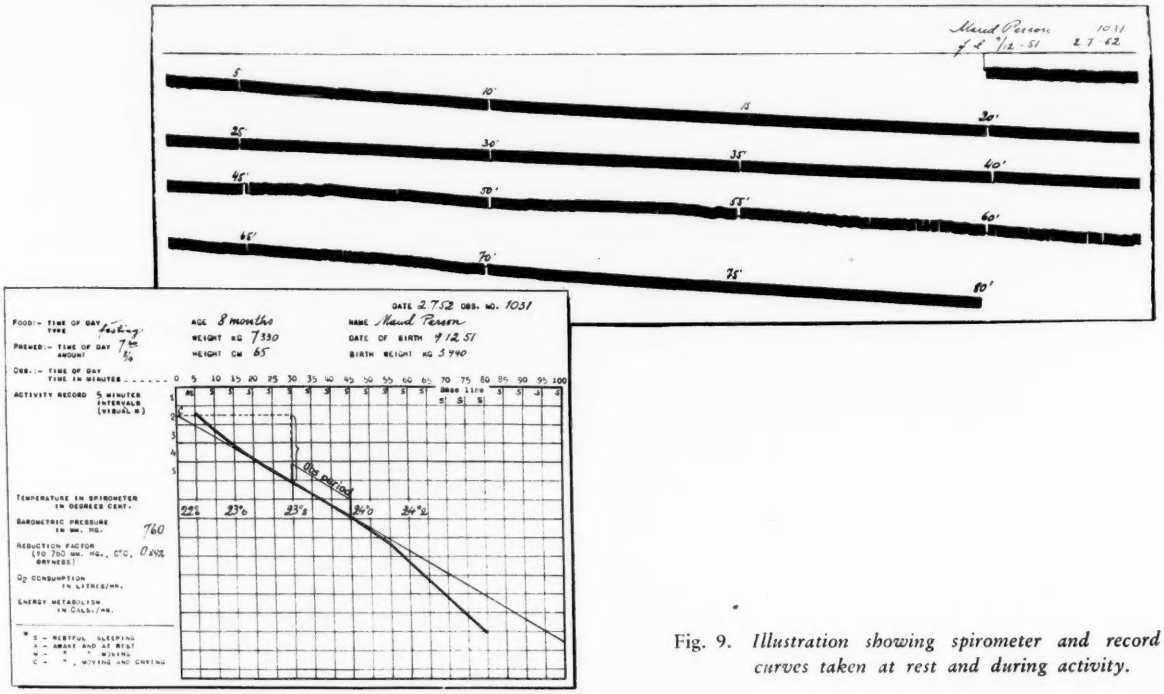

Fig. 9. *Illustration showing spirometer and record curves taken at rest and during activity.*

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By means of dividers, the distance from the basic line to the upper left corner of the notch on the kymographic curve at the end of every 5 minute period is set down at the corresponding 5 minute period on the record. (See Fig. 8). These points obtained are connected by a red line. It follows that this line will show the decrease in the volume of the system at 5 minute intervals. *In that part of the record in which an equilibrium in temperature, carbon dioxide and humidity is obtained in the system, the recorded decrease in volume equals the oxygen consumption of the child.*

When is the temperature and gas in equilibrium in the system?

In order to answer this question, it will be necessary to discuss various physical conditions in the system during the course of an investigation. As soon as the plastic cover is in position over the child and the spirometer filled with oxygen at the beginning of an investigation, the child is in an atmosphere which is identical to the room atmosphere, except for a certain increase in the oxygen concentration. The child begins at once to produce carbon dioxide, water vapour and heat, whilst inhaling oxygen. Thus the atmosphere around the child will change. The air pumped out at the beginning of the respiration chamber is slightly changed physically. At the same time carbon dioxide, moisture and heat, is accumulating in the respiration chamber, and oxygen decreasing. As the physical changes gradually become more and more accentuated in the atmosphere of the respiration chamber, an increasing amount of carbon dioxide, moisture and heat will be carried away from the respiration chamber at each stroke of the pump, until the products of metabolism removed from the chamber will equal the child's production.

In its passage the *carbon dioxide* is removed by the soda lime filter.

At first the *somewhat heated air* gradually raises the temperature in the different parts of the spirometer, and the spirometer in its turn gives off heat to the surrounding atmosphere. The

heat originates especially from the roof of the spirometer, which consists of a thin plate. As a result of the rhythmical movements of the pump, the spirometer is kept continually in motion and this produces a certain amount of air circulation outside. After some time, a balance should be struck between the raised temperature in the respiration chamber and the somewhat lower temperature in the spirometer, and the still temperature in the surrounding air, with its marked heat-absorbing capacity.

The *humidity* of the system will increase more and more, because there is no vapour-absorbing filter coupled to the system. The humidity will rise till it has reached a dew-point corresponding to the lowest temperature in the system, viz., in the spirometer. The continued production of moisture by the child in the system will result in condensation in the spirometer and thus a balance in humidity will be attained, if the temperature is constant. The humidity in the respiration chamber will be just short of complete saturation.

Once an equilibrium has been reached with carbon dioxide, temperature and humidity, the pump must be maintained at a constant capacity, and the temperature of the room remains unchanged during the progress of the investigation. Provided that the room is not too small and not sunny and that only one person is present, the temperature in the room, during the hours required for an investigation, does not change by more than a maximum of 0.5° C.

Control determinations. This behaviour of the temperature and the humidity has been checked by about 80 control determinations. By using thermo-couples at 5 minute intervals, the *temperature* has been measured in the spirometer, at the foot and head of the respiration chamber. At the same time in most investigations the skin and rectal temperatures of the child have been taken. The latter temperature has been obtained by means of two thermo-couples connected in series which were introduced to a depth of 5 cm. from the external sphinter ani. This thermo-couple accurate to within a ± 0.05° C. Regarding the depth for the temperature measurement in the ampulla of the

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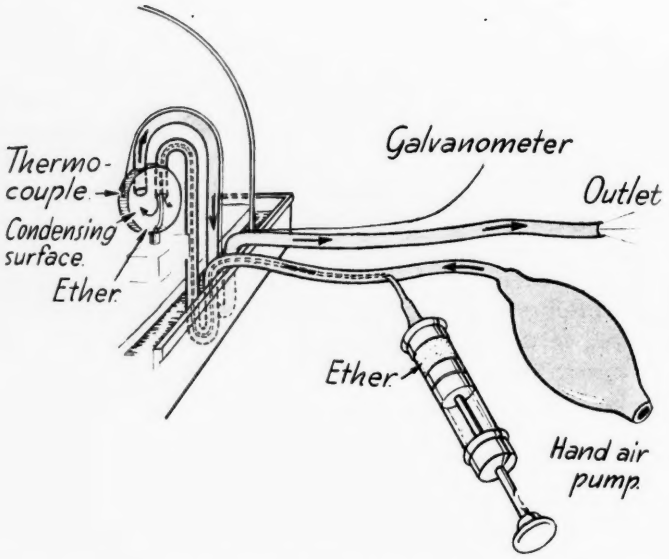

Fig. 10. *Apparatus for measuring humidity in the respiration chamber. Humidity is estimated by using the dew-point principle.*

A cylinder-shaped container with a diameter of 50 mm. is placed at the foot of the respiration chamber. One of the plain surfaces is coated with silver. Two tubes leave the container and pass through the water-trap to the outside of the respiration chamber. One to two ml. of ether is injected via a narrow tube into one of these tubes, and it then passes to the bottom of the container. A small hand pump is attached proximally to this tube shown in the diagram, and by means of this pump air passes through the tubes and aerates the container and thus causes the ether to vaporize. The temperature begins to fall, and as soon as condensation appears on the silver surface, the temperature is measured by means of a thermo-couple and this is taken to be the dew-point.

rectum, see *Karlberg* 1949. The *humidity* in the respiration chamber is measured by determining the dew-point. See apparatus for this measurements, Fig. 10.

A typical control experiment is illustrated in Fig. 11. One can

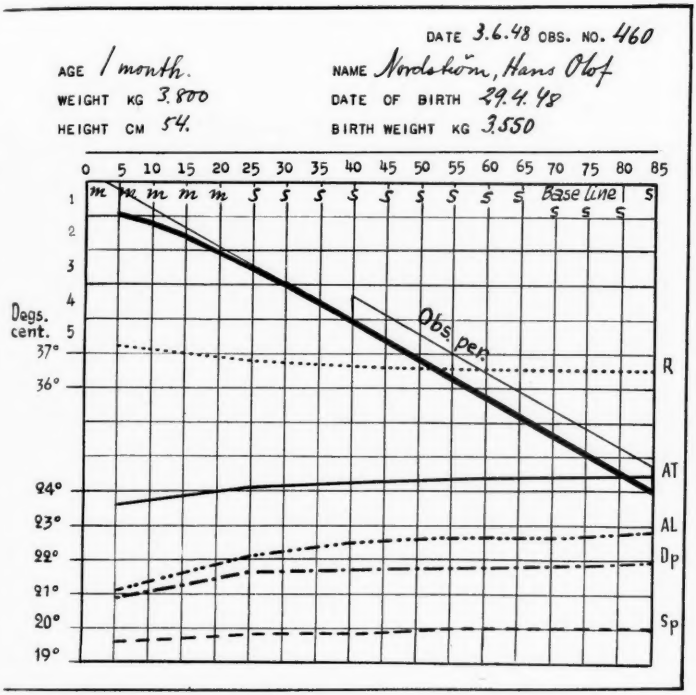

Fig. 11. *A typical record showing a control determination for temperature and humidity.* See corrections following page 10

see that after an initial rise in the temperature curves, they tend to level out, and at this stage gas equilibrium is becoming established. If the record curve is examined closely, it is seen that the curve tends to level out, as soon as the child is quiet, and the oxygen consumption is constant. The slope of the curve then increases gradually, until it commences to fall at a constant rate. This stage coincides with the levelling out of the temperature curves.

In the other control experiments, gas equilibrium was obtained in about 30 minutes, as judged from the temperature curves, provided the child settled down within 10—15 minutes after

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starting the investigation. If the child was active for a longer time, then gas equilibrium was attained later. The relationship between the record curve (= spirometer curve) and the temperature curves as shown in the typical control experiment, was the same in all the experiments. *Thus one is able to find in the record curve, the time at which gas equilibrium is present, i.e. where the record curve begins to fall at a constant rate, when the child is quiet.*

The length of the observation period.

Since the spirometer registers continuously the reduction in volume, there is no need to fix exactly the time for the initiation of the actual observation period during the course of an investigation. This can be calculated later. It is important that the child should be kept quiet for about 45 minutes in all in order that sufficient time is available to follow the slope of the spirometer curve. If a shorter time is employed, then the investigation becomes less accurate. For this reason, the observation period should be *about 30 minutes* for young infants. For more developed infants 20 minutes should suffice because they have a greater oxygen consumption.

In *earlier methods*, e.g. Benedict-Talbot's, the duration of the actual observation time had to be fixed during the course of an investigation. The same applies to American methods, using the open system, published in recent years. When using these methods, one must stop as soon as the child begins to move. In order to restart the investigation one has to wait until the child is quiet, and then one has to start all over again, as soon as the apparatus is readjusted.

With the *author's method* it is not necessary to stop the investigation, if the child commences to move. All one has to do is to ignore this period on the record, when one is making the calculations later.

Calculation of the oxygen consumption.

The spirometer curve and the record on which the curve of decreasing volume is superimposed are examined, and the period during which the child keeps still is defined. Shortly after the

commencement of this period, the curve becomes a straight line, and this period is taken as the observation period, i. e. when gas equilibrium is present. The incline of the record curve during the observation period is rendered more distinct by drawing a straight line along it and prolonging it upwards to the left. The incline of this line (a) is measured in mm./30 mts. as shown in Fig 11.

The same ruler or graduated triangle as used for measuring the calibration factor of the spirometer (c) is employed for this purpose. The decrease in volume per hour will then amount to:

$$\frac{a \cdot 60}{c \cdot 30} = \frac{a}{0.5 \cdot c} \text{ lits.}$$

In order to obtain the oxygen consumption, the volume has to be corrected to 760 mm. Hg., at 0° C. and dryness. This correction factor (r) is obtained from appropriate tables, e.g., Carpenter's "Tables, factors, and formulas for computing respiratory exchange and biological transformations of energy" (1924). In this connection, the barometric pressure and the temperature in the spirometer, during the observation period, are used because the decrease in volume takes place in the spirometer, and the humidity in the spirometer is 100 per cent.

The *oxygen consumption of the child* thus amounts to

$$\frac{a \cdot r}{0.5 \cdot c} \text{ lits./hr.}$$

Alcohol tests

In order to check an apparatus for indirect calorimetry, it is usual to perform alcohol tests. Many methods have been described in the literature, but they are essentially similar. A known amount of alcohol is burnt in the respiration chamber and the amounts of oxygen consumed and carbon dioxide produced are determined. Since these amounts can be calculated chemically from the amount of alcohol burnt it is possible to check the method. The most reliable control is obtained, as in the case of investigation of a quiet child, when the oxygen consumption and the carbon dioxide production are constant, i. e. when the administra-

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1. *Kymograph.*
2. *Alcohol burner.*
3. *Glass burette.*

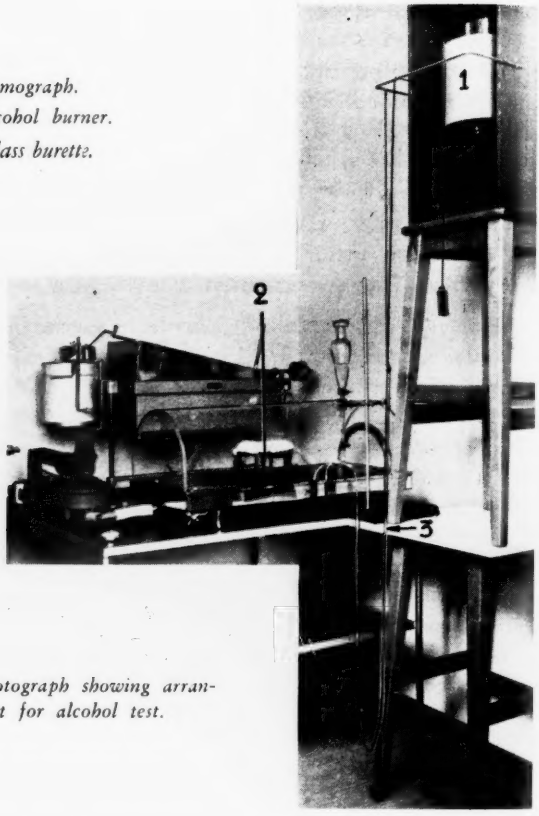

Fig. 12. *A photograph showing arrangement for alcohol test.*

tion of alcohol for combustion is constant. For this purpose various mechanisms have been described in the literature.

Author's method. The present method is based on the method recommended by *Benedict* and *Talbot* (1914). The arrangement see Fig. 12. When one uses three burette tubes which differ in size, an oxygen consumption of approximately 2.0, 3,6 and 5.0 lits./hr. respectively, are obtained, corresponding to an oxygen consumption in infants with a body weight of about 4, 8 and 12 kg. respec-

tively. The determination of the oxygen consumption is performed in exactly the same manner as in the case of children.

As the temperature conditions are likely to behave differently during the determination of oxygen consumption with an open alcohol burner, than when a child is concerned, the temperature is recorded with the thermo-couples placed in the spirometer, as well as in different parts of the respiration chamber. The humidity is not measured because condensation is found to form on that part of the interior surface of the plastic-cover where the temperature is lowest. The test takes about 60 minutes, and, as in the other investigations, the decrease in volume is estimated during the last thirty minutes. *The theoretical oxygen consumption* of the burnt alcohol is obtained from the formula: The combustion of 1 gm. of alcohol requires 1.459 lits. of oxygen.

Results. The results of these alcohol tests are present in Table 3. The oxygen consumption calculated for the last thirty minutes in different tests shows 10—12 per cent lower values, as compared with the theoretical amount. The spirometer curve, as shown in the record, becomes straight during the last 20 minutes of the test. Notwithstanding this the oxygen consumption is constant because the alcohol administration is constant during the test. A typical record, see Fig. 13. When the oxygen consumption is calculated exclusively from this part of the curve, the results become more comparable, and the difference amounts to 8—9 per cent (see Table 3).

When *the temperature curves* recorded simultaneously with the tests are studied, it will be seen that at no stage during the last 20 minutes temperature balance is achieved. A small distinct rise in the temperature is still to be found, but it is now practically constant. This explains why the gradient of the record curve has been judged to be constant (cp. the typical curve in Fig. 13). The rise in temperature during the last half hour in the spirometer, as well as in the respiration chamber (the mean value being 2—3 thermo-couples), and the volumes in the different parts of the system are used to correct the recorded decrease in volume during the last 30 minutes of the test (see Table 3). The values

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Table 5 *Alcohol tests*

Theoretic O_2 consump. calculated from 30 mts. obs. period. Lits. hr.	Found O_2 consump. calculated											
	From 30 mts. obs. period			From 20 mts. obs. period			From 30 mts. obs. period Corrected for temp. diff.			From 30 mts. obs. period corrected for temp. diff. and maisture diff.		
	Lits. / hr.	Diff: Found O_2 – Theoretic O_2		Lits. / hr.	Diff: Found O_2 – Theoretic O_2		Lits. / hr.	Diff: Found O_2 – Theoretic O_2		Lits. hr.	Diff: Found O_2 – Theoretic O_2	
		Lits. / hr.	Percentage		Lits. / hr.	Percentage		Lits. hr.	Percentage		Lits. / hr.	Percentage
5.00	4.44	— 0.56	— 11.2	4.44	— 0.56	— 11.2	4.80	— 0.20	— 4.0	5.00	± 0	± 0
5.02	4.52	— 0.50	— 10.0	4.59	— 0.43	— 8.6	4.88	— 0.14	— 2.8	5.08	+ 0.06	+ 1.2
5.82	5.42	— 0.40	— 6.9	5.43	— 0.39	— 6.7	5.80	— 0.02	— 0.3	5.96	+ 0.14	+ 2.4
5.90	5.30	— 0.60	— 10.2	5.30	— 0.60	— 10.2	5.74	— 0.16	— 2.7	5.96	+ 0.06	+ 1.0
5.92	5.30	— 0.62	— 10.5	5.30	— 0.62	— 10.5	5.72	— 0.20	— 3.4	5.96	+ 0.04	+ 0.7
6.02	5.48	— 0.54	— 10.6	5.53	— 0.49	— 8.2	5.90	— 0.12	— 2.0	6.12	+ 0.10	+ 1.7
Mean 5.61		— 0.54	— 9.90		— 0.52	— 9.23		— 0.14	— 2.53		+ 0.07	+ 1.17
No. 6												
3.00	2.66	— 0.34	— 11.3	2.75	— 0.25	— 8.3	2.92	— 0.08	— 2.7	3.06	+ 0.06	+ 2.0
3.09	2.62	— 0.47	— 15.2	2.71	— 0.38	— 12.3	2.94	— 0.15	— 4.9	3.12	+ 0.03	+ 1.0
3.12	2.64	— 0.48	— 15.4	2.70	— 0.42	— 13.5	2.86	— 0.26	— 8.3	3.04	— 0.08	— 2.6
3.10	2.74	— 0.36	— 11.6	2.84	— 0.26	— 8.4	3.00	— 0.10	— 3.2	3.14	+ 0.04	+ 1.3
3.64	3.20	— 0.44	— 12.1	3.28	— 0.36	— 9.9	3.48	— 0.16	— 4.4	3.66	+ 0.02	+ 0.6
3.80	3.34	— 0.46	— 12.1	3.44	— 0.36	— 9.5	3.64	— 0.16	— 4.2	3.80	± 0	± 0
3.62	3.22	— 0.40	— 11.1	3.30	— 0.32	— 8.8	3.50	— 0.12	— 3.3	3.64	+ 0.02	+ 0.6
3.60	3.24	— 0.36	— 10.0	3.32	— 0.28	— 7.8	3.50	— 0.10	— 2.8	3.64	+ 0.04	+ 1.1
3.62	3.20	— 0.42	— 11.6	3.34	— 0.28	— 7.7	3.46	— 0.16	— 4.4	3.58	— 0.04	— 1.1
3.64	3.40	— 0.24	— 6.6	3.40	— 0.24	— 6.6	3.56	— 0.08	— 2.2	3.66	+ 0.02	+ 0.5
3.62	3.28	— 0.34	— 9.4	3.34	— 0.28	— 7.7	3.58	— 0.04	— 1.1	3.72	+ 0.10	+ 2.8
3.62	3.40	— 0.22	— 6.1	3.40	— 0.22	— 6.1	3.60	— 0.02	— 0.6	3.70	+ 0.08	+ 2.2
3.66	3.40	— 0.26	— 7.1	3.46	— 0.20	— 5.5	3.58	— 0.08	— 2.2	3.68	+ 0.02	+ 0.5
3.64	3.22	— 0.42	— 11.5	3.26	— 0.38	— 10.4	3.52	— 0.12	— 3.3	3.66	+ 0.02	+ 0.5
3.66	3.22	— 0.44	— 12.0	3.38	— 028	— 7.7	3.52	— 0.14	— 3.8	3.70	— 0.04	— 1.1
Mean 3.50		— 0.38	— 10.87		— 0.30	— 8.68		— 0.11	— 3.43		+ 0.19	+ 0.55
No. 15												
2.00	1.85	— 0.15	— 7.5	1.85	— 0.15	— 7.5	1.92	— 0.08	— 4.0	1.96	— 0.04	— 2.0
1.99	1.81	— 0.18	— 9.0	1.81	— 0.18	— 9.0	1.92	— 0.07	— 3.5	1.99	± 0	± 0
2.01	1.84	— 0.17	— 8.5	1.84	— 0.17	— 8.5	1.97	— 0.04	— 2.0	2.04	+ 0.03	+ 1.5
1.98	1.80	— 0.18	— 9.1	1.80	— 0.18	— 9.1	1.93	— 0.05	— 2.5	2.00	+ 0.02	+ 1.0
1.97	1.72	— 0.25	— 12.7	1.72	— 0.25	— 12.7	1.84	— 0.13	— 6.6	1.89	— 0.08	— 4.1
Mean 1.99		— 0.19	— 9.36		— 0.19	— 9.36		— 0.07	— 3.72		— 0.01	— 0.7
No. 5												

values, viz., — 1 per cent to — 4 per cent. Here no attention is paid to the slight increase in absolute humidity that occurs with a rise in temperature. When a correction is made for this (see the last column of Table 3), there is a certain tendency to positive values, viz., — 1 to + 2 per cent. This faint positive tendency originates from the fact that the increase in humidity is calculated from the rise in temperature of the atmosphere in the respiration chamber, which, is somewhat high, seeing that the moisture in the respiration chamber during these tests is saturated and that it adjusts itself to the point with the lowest temperature, i. e. at the lower parts of the walls, or at the bottom of the respiration chamber. The exact amount may be found when these corrections are made.

The correlation between the noted and the theoretical values for oxygen consumption is good, viz., ± 1 to 3 per cent. When no correction is made for temperature and humidity and the oxygen consumption is calculated from the straight part of the spirometer curve, a difference of — 8 to — 9 per cent is obtained. This applies to alcohol tests.

Comparisons of alcohol tests and determinations on infants.

The temperature conditions in the system during investigations on infants and alcohol have to been compared.

When a litre of oxygen is consumed approximately the same caloric figure is obtained for the child and alcohol i.e. about 4.8 and 4.9 Cals. respectively. It follows that about the same amount of heat is produced in the system, if there is an identical level of oxygen consumption. However alcohol is burnt by using an open flame at a temperature of several hundred degrees centigrade, which causes a marked rise in the temperature of the immediately surrounding atmosphere, but the heat radiation is screened off above and below. *The child* gives off heat from a relatively big surface, which is at a temperature 5° C. — 7° C. higher than the surrounding atmosphere. At the same time the child is usually moving during the first 5—15 minutes

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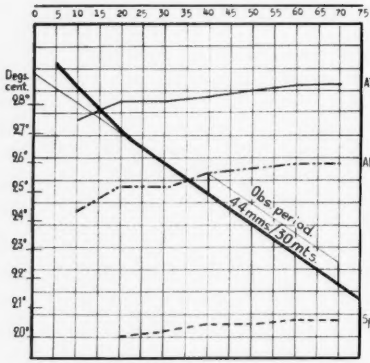

Fig. 14

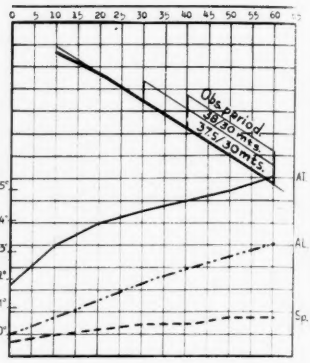

Fig. 13

Fig. 14. *A typical record of a child investigation showing temperature determinations in different parts of the system.*

Fig. 13. *A typical record of an alcohol test showing temperature determinations in different parts of the system.*

of the investigation. Accordingly, the heat production is high at the beginning but falls later. By comparison the alcohol flame remains constant all the time. Theoretically speaking, therefore, the time required to attain a temperature equilibrium should differ in these two instances.

The temperature curves show clearly that this is the case (cp. e.g.s. in Figs. 13 and 14). *In investigations on infants, temperature equilibrium is obtained earlier, than in corresponding alcohol tests.* In the latter instance, no temperature balance is achieved during the first hour, but gradually a practically constant rise in temperature occurs during the last 20—25 minutes of about 0.3 —0.5° C. and 0.4°—1.0° C. in the spirometer and the respiration chamber respectively.

Although heat equilibrium takes place more rapidly with infants, it is usually impossible to expect an *absolute equilibrium in temperature,* when the decrease in volume in the system begins to become constant, i. e. when the child is at rest. A certain slight rise in the temperature may occur, as seen from a careful examina-

tion of recorded temperature curves, though distinctly below that noted in alcohol tests, viz. up to about 0.3° C. in the spirometer, as well as in the respiration chamber (Fig. 14). This is probably unavoidable however, when one uses apparatuses based on the closed system even if there is more or less automatic regulation for temperature. This is obvious from accounts of alcohol tests in the literature, which use the closed system. *Levine* and his co-workers have, e.g., recorded a mean difference for oxygen value of — 3 to — 4 per cent.

An absolute temperature equilibrium cannot be obtained from investigations on children and adults, using a mouthpiece and a closed system. When comparing values obtained from his own spirometer and those from an apparatus using the open system, *Krogh* (1922), in fact, found the former to fall about 2 per cent below the latter. The error should be somewhat bigger with the author's apparatus for infants, because the volume of the system is bigger.

Conclusion. To judge from available reports of alcohol tests and temperature curves from alcohol tests, as well as from investigations on children, the systematic error of the method is not likely to exceed 3—5 per cent. Since the method is designed for clinical practice, a systematic error of this size should be acceptable, especially when the results of an individual investigation are compared with normal material using the same method.

Method of checking apparatus in clinical practice

When the calibration factor of the spirometer is determined, only regular checks of the soda lime and tightness are required. When this is done, the apparatus should be reliable. In clinical routine practice, there is no need for the much more complicated and laborious alcohol test.

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Chapter 3

INVESTIGATION CONDITIONS

The atmosphere in the respiration chamber

Respiratory exchange. As the child is placed in the respiration chamber, it is first exposed to ordinary room atmosphere. When the system is closed, the spirometer is filled with oxygen, i. e. about 7 litres of pure oxygen are added to about 50 litres of air. The purpose of this is to prevent *the oxygen concentration* in the system falling below the normal value i.e. 21 vol. %. The oxygen concentration in the system only falls to this level, when the child is consuming so much oxygen so as to bring the spirometer back to zero. (In actual fact it is just above zero because the volume increases slightly with the rise in temperature and humidity during the investigation). The investigation is discontinued. Thus the oxygen concentration in the system will diminish during the course of an investigation, from about 31 vol. % to 21 vol. %. This elevated oxygen concentration during an investigation has no effect, or any practical significance, on the oxygen consumption of a child.

The *concentration of carbon dioxide* will not, during the course of an investigation, exceed 1 vol. %, i. e. the highest limit at which it is advisable to keep it, e. g. according to *Levine* (1940). Even if an infant produces as much as 5 lits./hr. of carbon dioxide, the concentration of carbon dioxide will not rise above 0.56 vol. %, if the pump capacity is 15 lits./mt.

Heat regulation of the child. During an investigation *the clothing worn by the child* is approximately similar to that used in

the clinic, i. e. a shirt, vest, drawers or mantle or diaper and usually socks.

In the first place, *the temperature in the system* depends on the room temperature. In Swedish laboratories, it is about 18°—20° C. As previously shown (see page 43), a rise of 1°—2° C. in temperature occurs in the spirometer. During the investigation of the normal material which will be recorded below (see App. I), the mean temperature in the spirometer during the observation periods is kept at about 21° C. (ranging from 18°—23° C.). From the previously described control investigations on gas equilibrium in the system (see page 43), it was found that the temperature in the respiration chamber was 2°—3° C. higher than the temperature in the spirometer. The children have found themselves in an atmosphere of about 22°—26° C. during the observation periods. The humidity rises during the observation period and is about 90 per cent.

The somewhat elevated temperature in the respiration chamber, as well as the increased humidity, makes it more difficult to lose heat. At the same time the circulation of gases in the system will make it easier for the child to lose heat. From the results obtained from rectal temperatures in the control investigations, one sees a distinct decline in temperature of 0.1°—0.2° C. during the first half-hour. During the actual observation period it stays constant. Thus, heat does not accumulate in the child's body during an investigation.

Å. Gyllenswärd has studied by continuous registration the rectal temperature in infants while passing from wakefulsness into a light sleep. He examined them in bed and in a respiration chamber of the type used in the present work, and found that the same fall in rectal temperature occurred whether the child was in a bed or in a respiration chamber. (Personal communication.)

Conclusion.

The respiratory exchange of infants in the respiration chamber is therefore similar to that occurring in an ordinary room atmosphere.

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It should be possible to state that the heat evolved by a child during a metabolic investigation is not affected by the atmosphere in the respiration chamber to the extent of changing its body temperature.

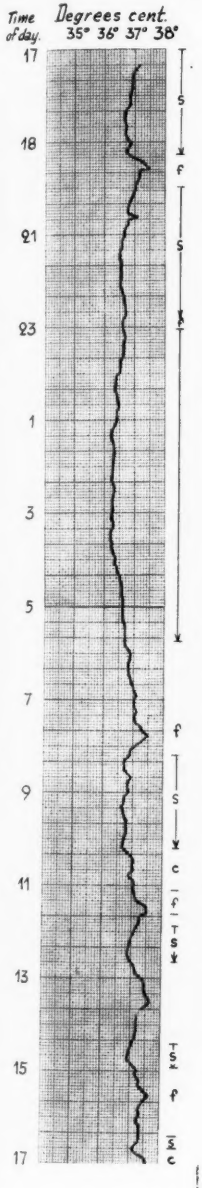
The oxygen consumption observed at investigations on infants with this apparatus should be similar to that of a child in a ward bed, other standard conditions being identical.

Basal or standard metabolism

The energy metabolism of an individual may vary very considerably within a short space of time, on account of various factors e.g. during activity and following food. The fact that it increases with a muscular activity, or during the hours following a meal, emerged as a result of the metabolic investigations made at the beginning of this century. Therefore, in order to compare the gaseous metabolism of an individual with that of others, the conditions should be as uniform as possible. From *adults and older children* the value is obtained in the morning when the patient is awake, fasting and unaffected by any muscular or physical disturbance. This value is called the *basal metabolism* of an individual, i. e. the various functions of the body are going on at a basal level. This can not be the true basal metabolism, because the energy metabolism, measured during a night's sleep gives lower values. This has been demonstrated, inter alia, by *Wang-Kern* (1928) and *de Bruin* (1936) on children between 4 to 10 years and 1 to 9 years respectively.

Helmreich (1924) is of the opinion that the "Grund-umsatz" is a relative quantity, being directly proportional to the administration of calories ta on individual, during the preceding days or weeks. (The investigation consisted of 6 older children. The full number and half the number of calories were used with all the patients. The emission of calories was based on the oxygen consumption, see text.)

It is also true that the level of the basal functions of the body are related to the body temperature, and it is a well known fact,



that the body temperature has a day and night rhythm. To show this day and night rhythm for infants *Å. Gyllenswärd* has very kindly placed one of his record curves for the body temperature at the disposal of the author (Fig. 15).

Apparantly the Dane *Krogh*, was the first to ~~question~~ the term *standard metabolism*. *Bierring* his countryman (1931), adopted this terminology in his investigation on boys between 7—18 years. *Du Bois* (1936) states in his monograph on "Basal metabolism in health and disease," that standard metabolism is the term that should be preferred, but owing to the difficulty in launching a new conception, he adheres to the term basal metabolism.

As far as *infants* are concerned, the term basal metabolism is even less to the point. The so-called basal conditions in connection with investigations on adults and older children cannot be applied to infants since they presuppose a conscious cooperation on the part of a subject. When an infants is fasting, i.e. hungry, is does not feel satisfied and expresses its dissatisfaction usually by moving and crying.

In order to secure as uniform conditions as possible in the case of infants, earlier investigators have adopted two particular lines. In the *American line*, introduced by Benedict-

Fig. 15. *A record of the rectal temperature in a 3 month old infant showing the day and night rhythm and the influence of sleep. (The record has been placed at the author's disposal by Å. Gyllenswärd.)*

Talbot (1914), and Murlin, the child was given a small meal immediately before an investigation, in order to quieten it and thus establish a physical quiescence at the cost a minor increase in metabolism as a result of the meal. The *German line* kept the subject fasting, though supplying some liquid and saccharin before an investigation, and in the event of uneasiness, a small dose of a barbituric acid preparation was given (Baer 1929, Schadow 1930—32). On the other hand *Schlossmann-Murschhauser* (1908—14) preferred to carry out their investigations on fasting children in the early hours in the morning when the children are usually asleep.

The last-mentioned method probably comes closer to true basal conditions than the others, but as a routine clinical procedure it is impracticable.

For the present investigation it has neither been possible to use true basal conditions nor the so-called basal conditions used for determinations in older children and adults. Therefore the standard metabolism appears to be the most appropriate one.

Suitable investigation conditions

In order to find the most suitable conditions for an investigation, one should be familiar with all the known factors regarding energy metabolism in infants.

The influence of muscular activity.

Muscular activity causes a definite rise in energy metabolism. Unfortunately it is not possible to estimate quantitatively the muscular activity. Several authors (see App. V) have tried to estimate the effect of muscular activity on energy metabolism and found an increase of about 40 per cent with moderate activity. In the course of the present investigation, increases as high as 80 per cent have been observed in cases showing marked agitation. If the energy metabolism of infants is to be compared, then it is necessary to have uniform conditions viz. that the child *must keep still immediately before and during the observation period.*

Therefore the investigator must be in a position to check the muscular activity of a child (see page 37 for procedure used in the present work).

The influence of sleep.

When an infant lies absolutely quiet, it usually keeps its eyes shut, and is in all probability asleep. It is difficult to make any definite statement regarding the effect of sleep, per se, on the energy metabolism of an infant.

It has been shown in older children, that the energy metabolism is lower during sleep, than in the morning on awakening e. g. *Wang-Kenn* (1928) *de Bruin* (1936). It would be natural to conclude that this difference in metabolism was due to the effect of sleep, but one must also consider that there may be a day and night rhythm for energy metabolism.

Å. Gyllenswärd (1951) was able to show in investigations on infants and older children that the body temperature was more markedly affected by a day and night rhythm than by sleep. It may be permissible to draw conclusions from the effect of sleep on the body temperature, which may be regarded as a manifestation of the total metabolism of a body. See Fig. 15, page 55.

Accordingly, *the dormant state* in which an infant almost invariably finds itself, as soon as it becomes quiet, *would appear to be of little importance* when one is investigating standard metabolism.

The influence of food.

After an ordinary meal, the energy metabolism increases and this is usually called "the specific-dynamic effect," reaching its maximum 1—2 hours after the meal, and then gradually disappears. Food rich in protein produces the greatest rise. All these facts can be found in text-books on metabolism (e.g. *Lusk* 1928, *Du Bois* 1936, *Peters-van Slyke* 1950).

"The specific-dynamic effect" on infants has been studied by several workers (see App. V), and more especially by *Levine*

and his co-workers. It is less marked in infants than older children and adults, and is less pronounced in young infants than more developed ones. The maximum increase in energy metabolism is said to be about 10—15 per cent after an ordinary mixed meal and the effect has disappeared mainly in about 3—4 hours.

When only a small meal is given, as in the case of the *American investigations*, the effect is less marked. It is natural to expect an error in the result when a meal is given, and one must now consider whether this error is constant or not. From the American observations one notes that a child does not always arrive at a state of quiescence immediately after a meal, and one may wait for ½ to 1½ hours. For this reason the influence of the food on the determined energy metabolism must vary with the time factor. In addition the so-called "specific-dynamic effect" varies with the age of the child. Therefore one may conclude that the error is not constant.

As stated by *Baer* (1929), the *German method* was as follows: The child is examined whilst fasting, and in order to calm it, the child is given some water or tea containing saccharin and a dose of a barbituric acid preparation.

The influence of a sedative.

According to *Bornstein-Holm* (1926), the energy metabolism is reduced by the usual doses of chloral hydrate, arsenic, bromide and morhpine, and especially by the first-mentioned substance. It was obvious that one could not use these narcotics.

After trying several such substances, *Baer* (1929) found that somnifene, a barbituric acid preparation (diethyl-diallyl-barbiturate of diethylamine) did not affect the metabolism, when given in doses of about 0.01 gm./kg. of body weight. He performed comparable investigations on 11 infants with and without somnifene, and obtained differences in values which were within normal physiological limits. Later *Schadow* (1930, 1932), *Essig* (1932) and *Mordhorst* (1933) used somnifene.

In *the present investigation* standard metabolism was deter-

mined in the morning after administering a barbituric acid preparation whilst the child was fasting.

The barbituric acid preparation consisted of *hexobarbytal* and was given in parts of tablets containing 0.25 gm. about 10—20 minutes before an investigation. The doses were based on the size of the child. In some cases the infants were so quiet prior to the investigation that a barbituric acid preparation was considered unnecessary. The doses were always the minimum necessary to achieve stillness, and as a rule, the children became quiet about 10—15 minutes after the commencement of the investigation, and remained quiet for the rest of the observation period. In this connection the stillness in the respiration chamber and the rhythmical sound of the circulation pump no doubt plays an important part. The slightest noise in the investigation room usually causes the child to move. The same effect is produced by tapping lightly on the plastic cover. When the children were taken out of the respiration chamber, they did not appear to be sleepy and behaved like children awaking from a normal sleep.

The condition of the children during that part of the investigation in which the oxygen consumption is calculated is defined as *superficial sleep.*

The *doses* used in the present investigation have been compared graphically with the weight of the child, as shown in Fig. 16. In order to see if a difference in dosage has any influence of the energy metabolism, the dose/kg. body weight for each child has been set out in relation to the difference between the actual and predicted values for energy metabolism. See Fig. 17. The predicted value is calculated from the mean relationship between the energy metabolism and the height and weight in all the normal investigation material. It would appear from the figure that a difference in dosage has no influence on the energy metabolism.

The influence of time of day.

In reports from earlier investigations, no particular attention has been paid to the precise hour at which investigations have

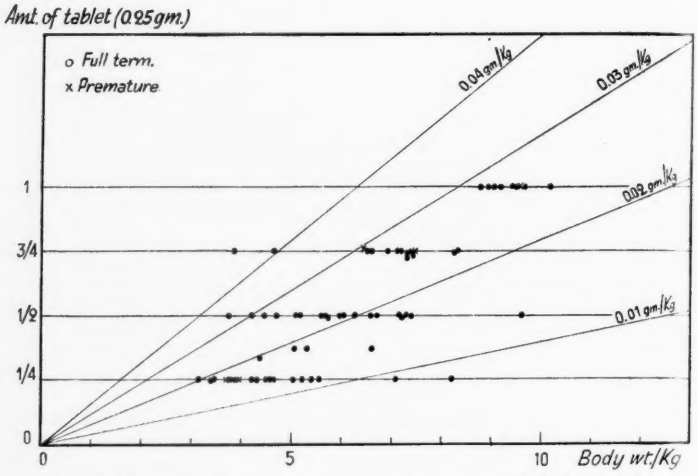


Fig. 16. *Relationship between premedication dosage and body weight.*

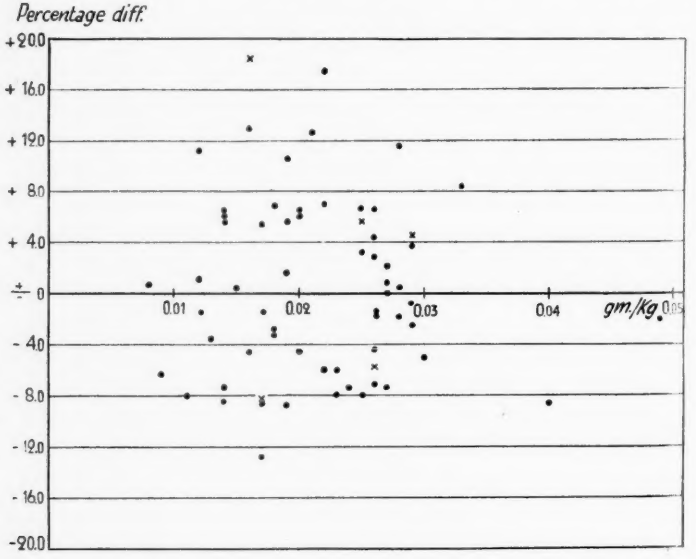


Fig. 17. *Relationship between the premedication/kg. body weight and the percentage difference between the actual and theoretical caloric value for the child.*

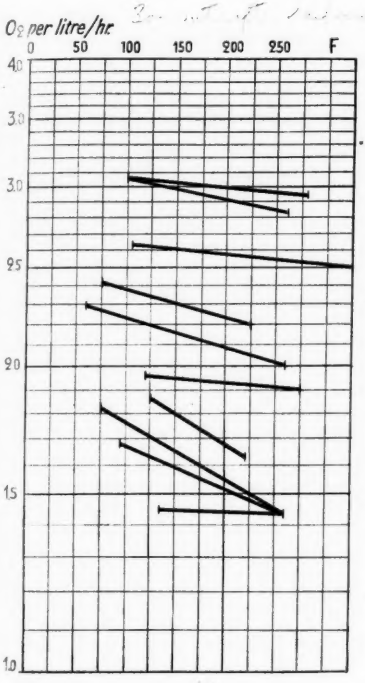


Fig. 18

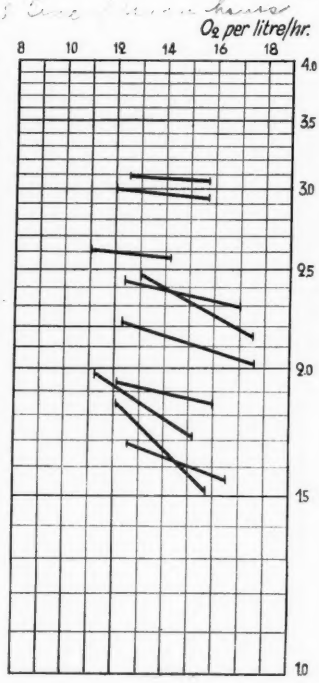


Fig. 19

Fig. 18. *A graphical record showing the influence of time of day on the energy metabolism. Two observations are made at different times during the day and are joined by a straight line.*

Fig. 19. *A graph showing the influence of food on the energy metabolism. Two observations are made on 2 occasions, at different times following feeding and are joined with a straight line.*

been performed. The occasions on which the actual time has been recorded in previous works, will be found in App. V. As seen in App. V the time of day was variable. It would appear from the literature that nobody seems to have clarified this point.

Author's experience. With the view of studying the possible effect of a day and night rhythm on the energy metabolism of children,

duplicate determinations were made on the same individual. From an extensive investigation material, cases were selected where determinations of oxygen consumption were made between 11 a. m. and 1 p. m. and again 4—5 hours later. Except for the time the test conditions were identical.

These times were chosen because bodily functions are usually maximal in the middle of the day. In all, 10 duplicate determinations were made on 8 infants. There was not more than 8 days interval between the individual determinations. On some occasions the first observation was made at noon and on other occasions 4 hours later.

The *results* are recorded in App. I, ~~App. II.~~ In Fig. 18 the results are compared graphically with the time of day. The two values of each duplicate determination are connected by a line. In all 10 cases, a higher value was obtained between 11 a. m. and 1 p. m., than 4—5 hours later. The probability of obtaining such an extreme result, if the time of day has no influence on the oxygen consumption, amounts to $2(\frac{1}{2})^{10}$, i. e. about 0.002 (signe-test).

The oxygen consumption of an infant will vary during the abovementioned hours, when the conditions are otherwise identical. *This suggests a day and night rhythm for the energy metabolism.*

Preliminary observations on the energy metabolism of older children (ages ranging from 4—8 years) at different times during the day and night, suggest that the energy metabolism has a day and night rhythm (*Gyllenswärd* and *Karlberg*).

Since the purpose of the present work is to determine the standard metabolism, the abovementioned investigations have not been pursued further. In conclusion it may be said that the time at which a standard metabolism test is performed is important. *In order to standardize the test conditions for determining the standard metabolism fixed times are used e. g. in the morning or early forenoon.*

Conclusion.

In conclusion, the conditions suitable for the determination of the standard metabolism can be summarized as follows:

Time of day. Morning or early forenoon.

Diet. An ordinary diet is given during the previous days. Prior to the investigation, no food is taken for a least 4—6 hours. The normal fluid intake is maintained by giving water or tea flavoured with saccharin.

Premedication. The child must keep still and in order to achieve this, a dose of hexobarbytal is administered in tablets containing 0.25 gm. The more developed infants receive ¾—1 tablet, and young infants ¼—½ tablet. Nothing is given to the smallest infants.

Chapter 4

INVESTIGATIONS EMPLOYED TO CHECK THE PRESENT METHOD

The random error of the method

In order to check the reliability of the method, duplicate determinations were made on the same children in a large number of cases. Similar conditions were used for the duplicate determinations i.e. both investigations were performed at the same time of day, and at the same time after the last meal, and the children received approximately the same doses of barbituric acid preparation on both occasions. The two investigations took place on different days but were always performed within 10 days of one another. 52 duplicate determinations were performed on 48 infants.

The *results* obtained are to be found in Apps. I, II, III, and the differences are shown in column 14. In fig. 20, these differences are compared graphically with the mean values for the respective duplicate determinations. In this connection, a distinction is made between values obtained with the small and with the standard plastic covers, and when one observation is made in the small chamber and the other in the standard chamber. As will be seen from Fig. 20 the differences are approximately of the same size, and relatively speaking, the accuracy grows the older the child becomes, which is to be expected. The error in measuring the incline of the record curve is the same and does not depend on the size of the decrease in volume. Moreover, the differences in temperature which may be present during an investigation will have a greater effect on the smaller decreases in

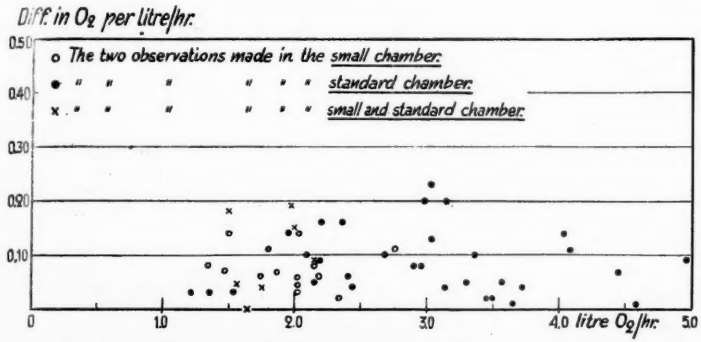


Fig. 20. *A graphical record illustrating the random error of the method. The difference between two observations on the same subject are recorded against the mean value obtained from the two observations.*

volume. As the differences obtained from the double determinations are of the same size, they have not been changed into percentage values. They have been statistically analysed according to *Dahlberg* (1940) and the differences are always based on the first and second observations in the respective duplicate determinations.

The mean difference = —0.00192 Number = 52
Standard deviation of the differences (δ_d) = ± 0.1039
The error in the mean difference = ± 0.0144
t = 0.133 P > 0.5

No significant difference is found between the first and second observations. Nor was this to be expected, because an infant does not require a preliminary test to become adapted to the investigation procedure as in the case of older children, where it is necessary to have the subjects cooperation in order to obtain a satisfactory result.

The error in an individual determination can thus be calculated according to the formula = $\frac{\delta_d}{\sqrt{2}}$ *The random error of the method therefore amounts to* ± *0.073.* In percentage values this would mean that if the oxygen consumption is 1 lit./hr. the random error is ± 7.3 per cent, and for 2.0 lits./hr. = ± 3.6 per cent and for 3 lits./hr. = ± 2.4 per cent and for 4 lits./hr. =

± 1.8 per cent. This random error is due to errors in the measurement of the volume, as a result of minor variations in temperature during the observation period, or to an erroneous interpretation of the incline of the record curve, or to errors in the measurement of the incline. It should include movements by the child that may have passed unnoticed during the observation period, and the individual differences in oxygen consumption, which may arise when the estimations are made on separate days.

The influence of food on the energy metabolism

In order to test the method, the well-known effect of an ordinary meal on the energy metabolism has been studied. This effect, i.e. the so-called "specific-dynamic effect" reaches its maximum about 1—2 hours after a meal, and returns again to normal about 4, 5 or 6 hours later. The most thorough study of this problem in infants has been performed by *Levine* and his co-workers (1927). He fixes the maximum rise at 8—10 per cent.

From an extensive investigation material, duplicate determinations made on the same individual have been selected, in which the oxygen consumption has been determined 1—2 hours after the last meal, and again after a lapse of 3½—6 hours. Ten of these duplicate determinations have been made. The test conditions were identical for the two observations. Both investigations have taken place on different days, with a time interval of 1—7 days. On some occasions the first observation was made 1—2 hours after the meal and on other occasions after 3½—6 hours. The results obtained are shown in Apps. I, II, and III column 15. The results are recorded graphically with the interval in time after the meal, and the two values for each duplicate determination are connected by a line (Fig. 19, page 61). In all 10 cases, the oxygen consumption was higher 1—2 hours after a meal than 3½—6 hours after a meal.

The probability of arriving at such an extreme result in the event of no difference being noted between the two periods equals $2(\frac{1}{2})^{10}$, i.e. approximately 0.002.

The effect of food on the energy metabolism can be ascertained by this method.

Chapter 5

METHOD OF CALCULATING THE ENERGY METABOLISM

In general

In the case of indirect calorimetry the energy metabolism is calculated either from the amount of oxygen (O_2) consumed, or from the amount of carbon dioxide (CO_2) produced per time unit. A prerequisite is a knowledge of *the caloric value of 1 litre of* O_2 *or of 1 litre of* CO_2 respectively, i.e. the number of calories resulting from an oxidation process consuming 1 litre of O_2, or producing 1 litre of CO_2 respectively. Carbohydrate, fat and protein give different caloric values on oxidation. The ratio between the amount of CO_2 produced and the amount of O_2 consumed, when measured in volume units per time unit, is called the respiratory quotient (RQ) and varies with the particular substance oxidised as shown below in Table 4.

Table 4.

Substance	Caloric value O_2 Cals./lit.	CO_2 Cals./lit.	RQ
Carbohydrate	5.047	5.047	1.000
Fat	4.686	6.629	0.707
Protein	4.485	5.599	0.801

The caloric values of CO_2 will be seen to vary more than the values of O_2. Normally a mixture of the three food-substances is

oxidized in the body, except on rare occasions. In order to obtain the exact caloric value, the interrelationship in the metabolism of these three substances must be known.

Besides the amounts of O_2 consumed and CO_2 produced, it is necessary to know the values for *urinary nitrogen* for a given period, because the urinary nitrogen indicates the level of the protein metabolism. Approximately, 1 gm. of urinary nitrogen is equivalent to 26.51 Cals., 9.35 mg. CO_2 (= 4.758 lits.) and 8.45 gm. O_2 (= 5.912 lits.) When proteins are metabolized the number of calories produced (p-cal), the amount of O_2 consumed (p-O_2) and the amount of CO_2 produced (p-CO_2) can be calculated from the urinary nitrogen. When p-O_2 and p-CO_2 are deducted from the total amount of O_2 consumed and CO_2 produced, the non-protein O_2 and non-protein CO_2 and non-protein RQ will be obtained, and this represents the mixed metabolism of carbohydrate and fat.

According to a method, originally devised by *Zunts* and *Schumberg* (1901), later modified and corrected by *Lusk* (1924), the correlation between carbohydrate and fat metabolism can be calculated from the non-protein RQ, and from this the caloric values of non-protein O_2 and non-protein CO_2. From non-protein O_2, or from non-protein CO_2, the non-protein calories will be derived, as well as the respective calories from the carbohydrate and the fat.

The sum of the calories of protein, carbohydrate and fat equals the total energy metabolism.

In 1924, *Michaelis,* a medical student, published an ingenious method for calculating the caloric value of one litre of oxygen representing the mixed metabolism of carbohydrate, fat and protein (see Fig. 21). From the relationship between the urinary nitrogen and the oxygen consumption per time unit, he calculates the protein metabolism as a percentage of the total energy metabolism. From this value and the total respiratory quotient, the total caloric value of one litre of oxygen is obtained (see the example in the nomogram).

Textbooks on this subject will give more detailed information regarding these problems e.g. *Lusk* (1928), *Du Bois* (1936), *Peters-van Slyke* (1950).

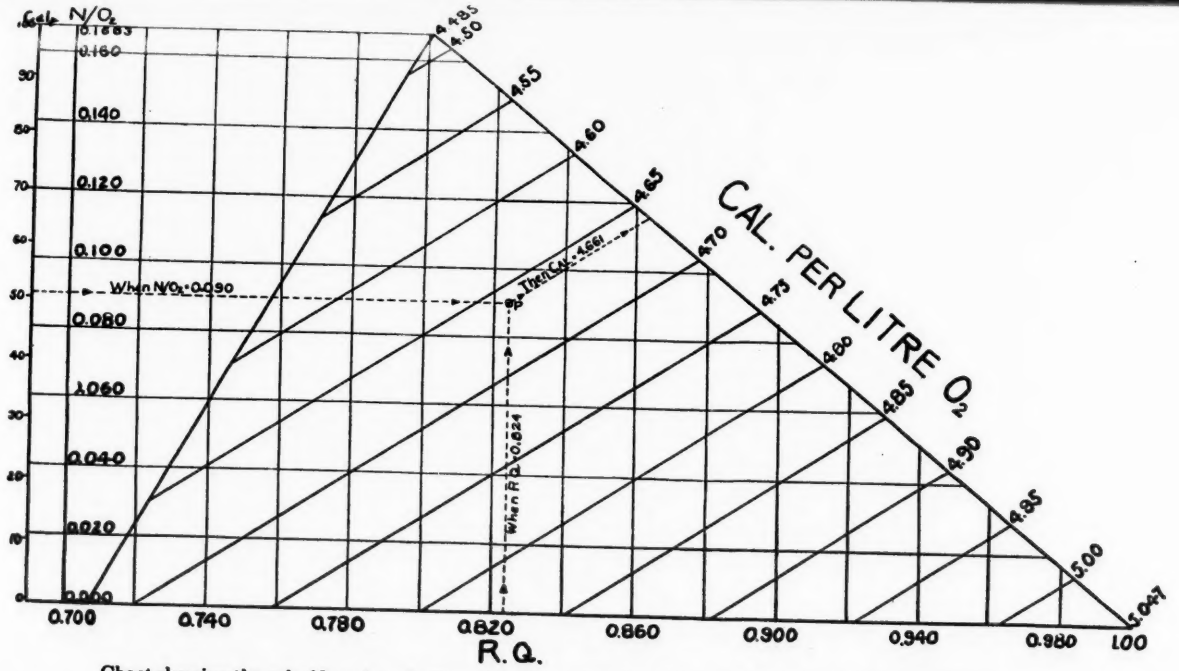


Chart showing the calorific value of a liter of oxygen according to the total R. Q. and the relationship of urinary nitrogen and total oxygen consumption.

Fig. 21. *Michaelis nomogram for calculating the caloric value of a litre of oxygen consumed. (J. Biol. Chem., 59: 51, 1924.)*

In standard determinations on infants

Protein metabolism in infants.

It is difficult to estimate accurately the amount of urine produced during a metabolic investigation. However, several authors have tried to overcome this (see App. V). *Levine* and co-workers paid particular attention to this problem. They found that protein metabolism constitutes about 15 per cent of the total metabolism. Apparently, the procedure was so complicated and difficult to perform that they were unable to prove the reliability of the method. In recent years they have ceased to use this method and have accepted the mean value of 15 per cent for protein metabolism in their calculations. The same applies to *Clagett-Hathaway* (1941) and *Benjamin-Weech* (1943). The author uses *the same mean value in the present work.*

The caloric value for O_2 and CO_2.

When protein metabolism is taken to be 15 per cent and the total respiratory quotient is known, the caloric values can be calculated from *Lusk's* table. The caloric value for oxygen is obtained very simply from *Michaelis'* nomogram.

Should O_2 or CO_2 be used for calculating the energy metabolism?

Both oxygen and carbon dioxide have been used in investigations on infants (see App. V). *Benedict-Talbot* used CO_2 for their calculations, because they consider the CO_2 determination much more reliable than O_2. *Murlin,* their contemporary, on the other hand, had access to a more accurate method for determining oxygen, and preferred to use O_2 for his calculations. The majority of later scientists used O_2 in their calculations including *Levine* and his co-workers. *Benjamin-Weech,* however, preferred the carbon dioxide method.

A considerable disadvantage in using carbon dioxide, is that the caloric value of carbon dioxide varies three times as much as the caloric value of oxygen, when the respiratory quotient changes. This has been illustrated graphically in Fig. 22.

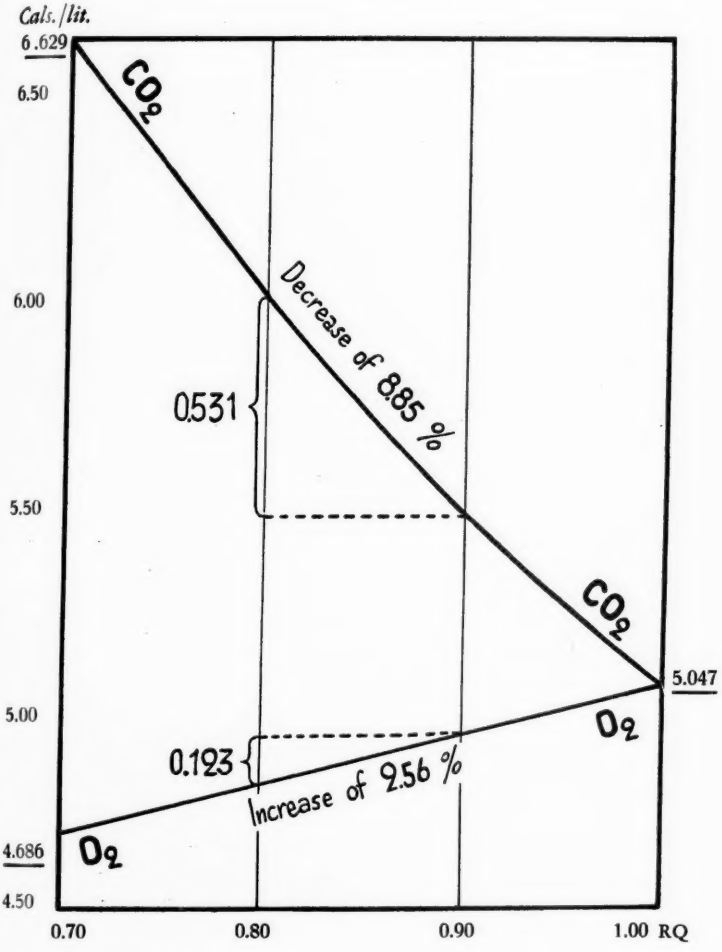


Fig. 22. *A graphical record showing the relationship between caloric value for O_2 and CO_2 and the respiratory quotient (RQ), and the difference between these two relationships.*

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Since *the respiratory quotient* is obtained by dividing the CO_2 by the O_2, and the random error of the methods used to obtain these values is at least ± 2 per cent, then the error in determining the respiratory quotient amounts to $\pm 2\sqrt{2} = \pm 2.8$ to 3 per cent. If the respiratory quotient is found to be 0.85, its 95 per cent confidence interval is 0.80—0.90. This implies that in estimating the caloric value for carbon dioxide there is an error of about ± 4.5 per cent, whereas with the oxygen it is about ± 1.25 per cent, (see Fig. 22). *Therefore, when the carbon dioxide and the oxygen are determined with the same exactness, the oxygen should be preferred for calculating the total caloric value.*

If one wishes to simplify the method for determining the energy metabolism by using *only one* of the two factors, then *oxygen* should be used. This has been done by the author.

The calculations, when only O_2 is determined.

When oxygen is determined alone the caloric value of oxygen has to be calculated from a *mean value for the respiratory quotient.*

The literature offers a great many determinations of the respiratory quotient in infants (see App. V). They have been summarised in Fig. 23, and the children have been classified according to age, as shown in the diagram.

Within each group, a distinction has been made between the results obtained by different authors. In the more extensive materials the mean values for each age group have been calculated and shown graphically. As will be seen from Fig. 23 there is a distinct tendency to a reduction in the respiratory quotient during the first days of life. However, after the first week of life, no definite variation is seen. The mean values for the period between the second week and one year have been recorded in Table 5. The mean values for the German authors as well as those of Garot, show a tendency to fall below the values obtained by the Americans. The explanation, at least as far as the Germans are concerned, may be due to a systematic error in determining the amount of carbon dioxide produced (see the account of Knipping's apparatus on page 25).

Table 5. *A table summarizing Fig. 21 showing the number of RQ observations in different age-groups and the mean RQ value for different authors.*

Age group months	Benedict-Talbot No.	Flemming No.	Levine et al. No.	Benjamin-Weech No.	German authors No.	Garot No.
½—1	7	1	6	—	20	—
2—3	24	23	5	—	10	4
4—5	78	19	14	7	—	9
6—7	37	35	13	42	—	7
8—9	18	8	7	35	—	3
10—11	10	1	1	41	1	—
Total:	174	87	46	125	31	23
RQ mean value	85.93	87.44	87.80	85.03	79.23	81.35

From the diagram in Fig. 23 and the values given in Table 5, *the mean value of the respiratory quotient* from the second week to one year of age can be said *to be about 0.86.* When the protein metabolism is taken to be 15 per cent, then the *caloric value of one litre of oxygen consumed is 4.825 Cals.*, and this is most readily obtained by using Michaelis' nomogram (page 69). This is a useful value, because exactly the same value is used in investigations on adults and older children.

The theoretical error in using 4.825 Cals. From a study of the diagram in Fig. 23, the 95 per cent confidence interval for the mean value of 0.86 for the respiratory quotient, may be taken to be 0.96—0.76 in an individual determination.

This deviation is due to the random error of the method in determining the respiratory quotient, which is calculated to be about ± 0.05, and is due to the individual variations. In spite of these relatively marked differences in the respiratory quotient, the corresponding variations in the caloric value of oxygen does not exceed ± 2.5 per cent (see Fig. 22).

With the method used in the present investigation, where the caloric value is taken as 4.825 Cals., the error should be smaller, because the variation in the respiratory quotient which would arise from the random error of the method in determining the

respiratory quotient is eliminated. When the variation in the respiratory quotient is estimated at ± 0.05, the error in determining the caloric value of oxygen amounts to about ± 1.3 per cent. *Even if it rose to ± 2 per cent, such an error would be of little practical significance in clinical determinations of standard metabolism.*

The calculation in the present work

4.825 Cals. is taken as the caloric value of 1 litre of oxygen consumed. The mean value of the respiratory quotient is fixed at 0.86, and the protein metabolism at 15 per cent.

The standard metabolism is assessed in *Calories per hour*, rather than in Calories per 24 hours, as earlier investigators often preferred to do. Calories per hour is the unit of measure used by the more recent workers. This seems sensible, because an infant can hardly live under standard conditions for 24 hours.

Thus, the calculation of the primary values of the investigations (see page 45) will look as follows:

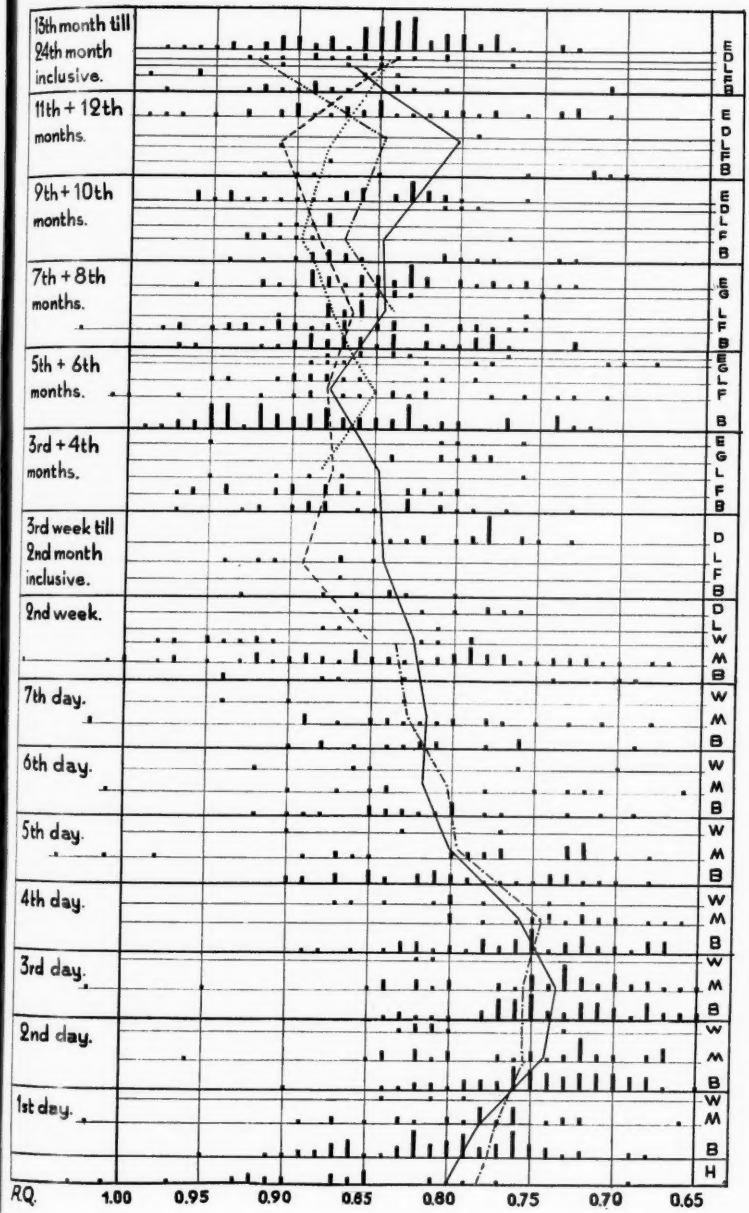
$$\frac{a \cdot r \cdot 4.825}{0.5 \cdot c} \quad \text{Cals./hr.}$$

a = the decrease in volume in the spirometer expressed in mm. per 30 minutes.

Fig. 23. *Respiratory quotients as described in the literature are summarized graphically.*

H = Hasselbalch — *D = German authors*
B = Benedict-Talbot — *F = Flemming*
M = Murlin et al. — *G = Garot*
W = Weiss — *E = Benjamin-Weech*
L = Levine et al.

———— *represents Benedict-Talbot's plotted mean values for each age group*
. — . — . — » *Murlin et al.'s* » » » » » » »
- - - - - - » *Levine et al.'s* » » » » » » »
. . . . . » *Flemming's* » » » » » » »
. . — . . — . . » *Benjamin-Weech's* » » » » » » »


13th month till 24th month inclusive.
11th + 12th months.
9th + 10th months.
7th + 8th months.
5th + 6th months.
3rd + 4th months.
3rd week till 2nd month inclusive.
2nd week.
7th day.
6th day.
5th day.
4th day.
3rd day.
2nd day.
1st day.
E D L F B
E G L F B
D L W M B
W M B
W M B H
RQ.
1.00
0.95
0.90
0.85
0.80
0.75
0.70
0.65

r = the correction factor for barometric pressure, temperature and moisture.

c = the calibration factor of the spirometer.

Since only *a* and *r* vary from one investigation to another, the formula

$$a \cdot r \cdot k$$

will equal Cals./hr., k is constant.

Chapter 6

RANGE OF APPLICABILITY OF THE PRESENT METHOD

As stated above, the present method has been devised in order to obtain a more simple method for estimating *the standard energy metabolism in infants.* It is necessary for the subject to be below a certain age or, more correctly, below a certain maximal size, when using this apparatus. It has been designed for *children up to one year of age,* but considering that growth is not very rapid at one year, the apparatus can be used for investigations on children above one year, and even up to 2 years of age, if the body size is suitable.

There is no definite lower limit for the size of a body, but as shown in the calculations for the random error of the method, the error increases as the child becomes smaller (see page 66). In order to make sure that the results are reliable, *2.5 to 3.0 kg. body weight* are taken as the lower limits.

Further, the children investigated are supposed to have a *respiratory quotient which varies within normal limits,* because the oxygen consumption is the only determination made and a mean value for the respiratory quotient is used for calculating the energy metabolism. The child must be kept on an ordinary diet during the days immediately preceding an investigation. When investigating an infant in the first week of life, the accuracy of the calculated caloric value diminishes, owing to the fact that the respiratory quotient, shows a distinct tendency to vary at this time of life (see page 72).

Conclusion.

The apparatus can be used for determining the standard metabolism of infants between 1 week old and 1 year, and even up to 2 years if body size permits. The children are supposed to have a respiratory quotient which varies within normal limits.

Chapter 7

THE AUTHOR'S INVESTIGATION OF THE STANDARD ENERGY METABOLISM

The age limits of 1 week to 1 year have been adopted for the following reasons. Children in the first week of life have been excluded, because during the first days of life they appear to have a lower energy metabolism than in the second and third weeks and later. This was shown by *Benedict-Talbot, Murlin* et al. *and Marine* et al. (see App. V). The increase in the energy metabolism of newborns takes place gradually. But according to Benedict-Talbot, whose newborn material is more extensive than any other, an infant reaches the normal energy metabolism at the end of the first week. For this reason, *the lower limit* is taken to lie between the 7th and 8th day of life. *The upper limit* is fixed at one year, because children older than this can not fit into the present apparatus.

Material

60 normal and healthy full term infants with ages ranging from 8 days to 12 months have been investigated. 33 were boys, 27 girls. In addition, 5 older prematures, all boys, with a body-weight in excess of 3 kg have been included. In the analysis of the material the latter have been separated from the others.

Fig. 26 shows the distribution of the material in the different age, height and weight groups.

Criteria of normality.

None of the children have revealed any signs of previous disease, e.g., infective diseases or nutritional disorders. The mental development has been observed whilst making investigations on the infants, as well as by the nursing staff in the wards or in the children's homes. All observations were negative. The physical development of the children was checked by using a swedish growth chart.

Swedish growth chart.

As far as infancy is concerned, the chart is based on various body measurements on Swedish children by the present author in conjunction with *A. Perman* during the years 1950 and 1952.

These investigations consisted of 1.600 children with ages ranging from 2 weeks to 2 years.

The measured children may be said to be a fair representation of Swedish infancy. The children were healthy and were examined at well baby clinics, before, during and after the measurements. The growth chart has been elaborated in association with *S. Iggbom,* and it was compiled by comparing the age-height and height-weight in a logarithmic coordinate system.

Preliminary reports are submitted at an exhibition at the Tenth Northern Pediatric Congress in Stockholm in 1951 (see a brief account of the proceedings published in Acta Paediatrica, Supplement 83). A more detailed report of this work will appear later in Acta Paediatrica.

Physical development of the present infant material. When the age, height, and weight of each individual are known, the physical development has been checked by plotting the values on the growth chart (see Fig. 26). As will be seen, all the cases, except one, fall within the 95 per cent confidence interval, as far as the correlation of height-weight are concerned, i.e. the external physical development. Only 4 out of 60 cases lie outside the 95 per cent confidence interval for age and height. The youngest prema-

tures fall outside, as was to be expected. Thus, the full term infants in this material are found to correspond, in the matter of physical development, to the findings obtained with normal Swedish infants.

Conclusion.

The full term infants can be regarded as healthy and normal, and the 5 prematures as healthy.

Experiments

In the author's material the *oxygen consumption* was determined according to the previously stated methods.

The investigation conditions were the same as those described as being the most suitable in routine clinical practice (see page 62): The determinations were made in the morning or early forenoon, and the children were fasting. The older infants had their last meal the evening before and the younger not later than 4—6 hours before an investigation. During the previous day the infants were given an ordinary adequate diet. In about half the number of cases the children were given weak tea containing saccharin before the investigation. A barbituric acid preparation (hexobarbital) was administered for the purpose of calming the children during the investigation. (For dosage see Fig. 16, page 60).

After the investigation the children were *weighed* naked and their *heights* measured. The children lay at full length and were measured from the top of the head to the heels.

The *standard energy metabolism* was calculated from the oxygen consumption, by using a mean value of 15 per cent for the protein metabolism, and a mean value of 0.86 for the total respiratory quotient.

Results

The results obtained from the investigations have been arranged in a tabular form (see App. I). In *each individual case*, the table gives the following particulars: a description of the

child (sex, weight at birth, age, present weight, and height), and the type of chamber used, the temperature in the spirometer, the time interval following feeding, the premedication, the time of day, and the oxygen consumption in lits./hr. and the calculated energy metabolism in Cals./hr. (also Cals./24 hrs.).

The same table includes the duplicate determinations for calculating the random error of the method, for studying the influences of food and for studying the influence of the time of day. These duplicate determinations have been accounted for previously. As a rule, they have been performed on the children that formed, part of the material for determining the standard metabolism. The energy metabolism has been calculated only for such observations that have been included in the later material (see columns 17 and 18 in the table).

For *each individual*, the calculation of the standard metabolism has been made from *one observation only*.

The influence of sex.

In order to form an opinion of the influence of sex, the resulting caloric values have been plotted in relation to the body weight (see Fig. 24). This diagram does not suggest any tendency to a sex difference. Accordingly, no statistical analysis has been undertaken.

Analyses of extensive materials in the literature have failed to disclose any significant difference between the sexes (*Benedict-Talbot* 1914, 1915 and 1921, *Levine-Marples* 1931, *Janet-Bochet* 1933).

Consequently, no distinction has been made between boys and girls in the analysis of the material.

The correlation of standard metabolism to body size.

The diagram in Fig. 24 shows a distinct correlation between the caloric values and body weight. It is also an old and well-established fact that the energy metabolism is closely related to the size of an individual. In order to compare the standard metabolism of different individuals, the values have to be considered in relation to the size of the individual.

A large number of correlation factors representing the body

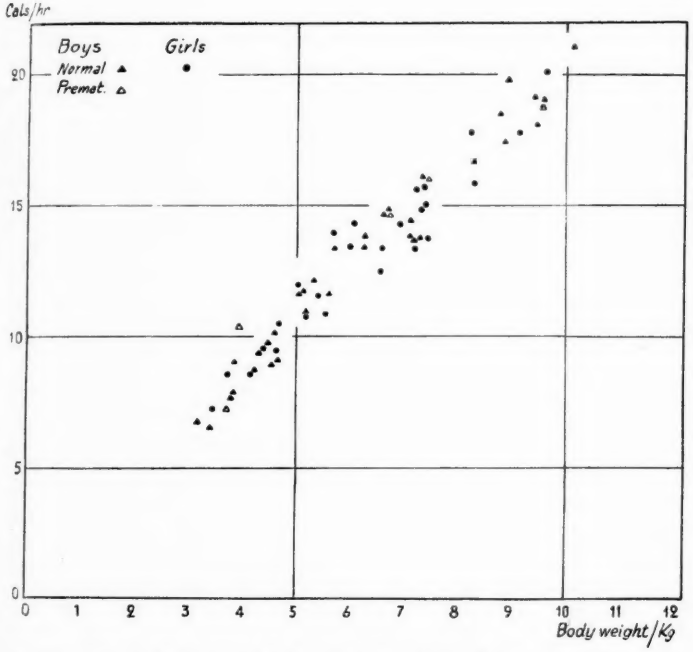


Fig. 24. *The author's material showing the relationship between the energy metabolism and the body weight.*

size have been tried, but not one of them has been found to be superior. These factors are obtained from one or several measurements made on the child. Since the purpose of the present work is to obtain a practical method for use in clinical work, these measurements must be obtained easily. Most clinics use the age, weight and height, when describing the physical development of a child. It would be an obvious advantage if the age, height and weight were sufficient to represent the body size. Accordingly, they will be examined.

Chapter 8

CORRELATION FACTORS USED IN THE LITERATURE

Body weight.

The body weight has been used often, and in fact it is the most common correlation factor used in metabolic determinations on infants. Practically every author has adopted the formula of Cals./kg., either alone, or in comparison with other correlation factors. However the stated mean value of Cals./kg. has varied from one material to another ranging from about 43 Cals./kg. to about 60 Cals./kg.

These variations may be due to differences in materials or in methods or to the fact that the investigated children were of different ages. When studying diagrams in which the calories are plotted against the body weight in the different materials, the regression lines are found to be curvilinear against the weight axis and not rectilinear. This curve commences at 8—10 kg. (e.g., *Benedict-Talbot* 1921, *Janet-Bochet* 1933). This tendency becomes more pronounced with children more than 1 year old (see e.g., *Benedict-Talbot's* comprehensive child material in their monograph "Metabolism from birth to puberty," 1921, from which Fig. 25 depicting Cals./kg. for boys has been borrowed. However, *Benedict-Talbot* still maintain that the body weight is the best correlation factor for determining the standard values for infants, and for the period immediately following infancy. However, the value of Cals./kg. must be altered for different ages even in infancy, and this has been done in the standard tables published by *Benedict-Talbot.*

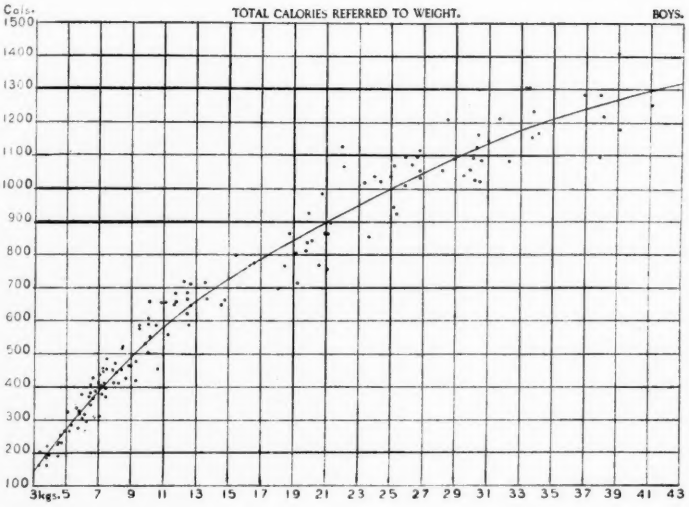


Fig. 25. *Basal heat production of boys per 24 hours referred to body-weight. Benedict, F. G., Energy requirements of children from birth to puberty. (Boston M. & S. J., 181: 107, 119.)*

The body height.

The height has been used in many of the more extensive investigations, although it is never used in preference to the weight. One reason for this is, that it is easier to fix the weight of an infant than the height.

Body surface area.

When metabolic research was initiated first, attempts were made to find a common correlation factor for different ages. *Bergmann* (1848, 1852) tried to explain the relatively bigger food demands of small animals, as compared with large animals of the same species, by the generalization that the heat-production of a body is proportional to its surface area. *Bergmann's* work was entirely comparative and theoretical.

Meeh's formula. Rubner adopted this suggestion and introduced it into practice, (first publication 1883). He formulated a hypothesis, called *Rubner's law* to the effect that the energy metabolism of an individual, originally calculated from the production of carbon dioxide, is directly proportional to the body surface, according to *Meeh's* formula (1879), viz., the surface equalling $k \cdot W^{2/3}$. He stated a general value of 1,000 Cals./24 hrs./sq.mt. It was suggested that this should apply irrespective of body size.

The constant in Meeh's formula, i.e. originally 11.9 was obtained from material consisting of 2 infants and the rest mainly adults, and this has been modified by later scientists who have given values from 8.5 to 12.9.

As far as infants are concerned, values of 10.3 (*Lissauer* 1903), 12.3 (*Howland* 1911), 10.0—10.5 (*Phaundler* 1916) and 10.0—10.1 (*Benedict-Talbot* 1914) have been noted. Apparently, it has not been possible to find a common constant for the whole period of infancy. It has varied, on one hand, with the age, and, on the other hand, with the nutritive condition of the children.

However all of them have used the *weight in a power of* $\frac{2}{3}$, i.e., precisely the power applied in order to derive a 2-dimensional surface from the 3-dimensional weight.

Many authors realized that a calculation of the body surface according to Meeh's formula was only approximate.

Howland-Dana's formula. In the opinion of Howland-Dana (1911), it was not satisfactory in the case of infants. They arrived at a correlation of $y = 0.483 \cdot w - 730$ (y = surface in sq. cm., w = weight in gm.). They confessed to some hesitation with regard to the constants.

Du Bois formulae. When the body surface is obtained from the weight alone, all calculations must be of questionable accuracy in an individual case, because the external proportions of the body can vary so much and it is not possible to allow for them in such a formula.

On the basis of careful direct measurements made on several individuals, Du Bois-Du Bois (1915) worked out the *linear formula*, based on a number of body measurements. Since this procedure was too elaborate, Du Bois worked out a formula using

the height and weight as the only variables (Du Bois-Du Bois 1916, Sawyer-Stone-Du Bois 1916).

Height-weight formula: $A = W^{0.425} \cdot H^{0.725} \cdot 71.84$.

The formula was derived from careful direct measurements on 10 individuals. The exponents for weight and height have been mathematically worked out so as to render the surface 2-dimensional. Originally, this formula was to be used for adults and older children. However, *Hannon* has devised a nomogram for infants, the formula being used in its ordinary shape (quoted by Du Bois 1936). *Unfortunately the material that forms the basis for this formula does not include any infants.* Only one small child was actually measured. She was a poorly nourished girl, aged 21 months and weighing 6.27 kg., i.e. the average weight for an infant of 4 months. In spite of this, the nomogram has since been used in various connections for calculating the body surface of infants.

Edith Boyd's formulae. In 1935 Edith Boyd published a monograph for determining the body surface area. From a comparison of all commensurable values recorded in the literature, she produced new formulae, viz., one in which weight alone serves as a variable, another with weight and height, and in both age enters as a function in the exponent for the height. She calls the formulae self-adjusting.

However none of her formulae have been much used in metabolic studies on infants.

Discussion: Rubner's law was applicable to adults and various animals, but was less satisfactory for infants. In fact, its theoretical basis was subjected to criticism at an early date.

One of the first critics was *Kassowits* (1904, 1913). In his opinion, Rubner's law was nothing but a mathematical coincidence. He contended that the energy metabolism could not be proportional to the body weight in a power of $\frac{2}{3}$, on account of the increased discharge of heat per unit of mass for relatively small animals, seeing that this mathematical coincidence also applies to cold-blooded animals.

Rubner's views on the discharge of heat began to be less accepted. On the other hand his law was accepted as being applicable to the general rule that the energy metabolism is proportional to the weight in a $\frac{2}{3}$ power *Hoesslin* (1888), *Pirquet* (1917), *Phaundler* (1916), *Stoeltzner* (1928/29). In a summary, of experiences recorded in the literature up to 1931 regarding this surface rule, *Helmreich* concludes that it does not affect newborns or infants, but only individuals above 1 year of age.

In spite of the fact that the body surface, estimated according to *Meeh's formula,* with a more or less modified constant, has been used by most workers on energy metabolism in infants, it will seem never to have proved entirely satisfactory, with its inadequate theoretical basis and its varying values for Cals./sq.mt. during infancy (see among others, *Benedict-Talbot* 1921).

The body surface area, calculated from *Hannon's nomogram* of the *Du Bois' height-weight formula* has been used by several workers studying the energy metabolism of infants in recent years, as well as other correlation factors. It would appear that it has not proved definitely superior to other correlation factors on any occasion. Its chief advantage would seem to be, that if height and weight are both used, then the variations in nutritive conditions should be levelled-out.

More than one factor.

According to Kestner-Knipping. Their standard values are based on a combination of a *weight factor* and an *age factor.*

In a monograph, entitled "Die Ernährung des Menschens", they published for the first time their normal values for infants, and these were tabulated up to adult age. The values for adults are said to originate from *Harris-Benedict's* formulae[1] and have been applied by the authors to children and adults below 21 years of age, including infants.

[1] *Harris-Benedict's* formulae for adults from 1919.
For males: $h = 66.4730 + 13.7516 \cdot w + 5.0033 \cdot s - 6.7550 \cdot a.$
For females: $H = + 655.0955 + 9.5634 \cdot w + 1.8496 \cdot s - 4.6757 \cdot a.$
h = heat production.
w = body weight in kg.
s = stature in cm.
a = age in years.

Only body weight and age are used as correlation factors for infants, boys and girls being dealt with separately. The *weight factors* will be found from the first half of *Harris-Benedict's* formula for adults, i.e. for the boys 66.4730 + 13.7516 · w and for the girls 655.0955 + 9.5634 · w. To this weight factor is added an *age factor*, calculated according to age in months, with intervals as big as 2 months. Kestner-Knipping do not state how they arrived at this age factor. An attempt at analysis shows that, as far as boys are concerned, the factor is directly proportional to the mean height of the different age groups. It would appear in the case of girls that it is not possible to obtain such a simple relationship between the mean height and age factor. In addition, the girls' age factor is always negative. It begins with a negative figure, which decreases successively, to compensate for the big standing constant of 665 calories for females in Harris-Benedict's formula.

The literature provides no clue to the question as to how *Kestner* and *Knipping* came to establish the two variables for the relationship between the energy metabolism and the weight and age. Nor do these two authors give any original results.

However as the standard values have been calculated it can hardly be regarded as correct to use a part of a multiple regression equation from a statistical analysis of adult material for values in infants.

According to Levine-Marples. Their normal values for infants (1931) are based on multiple regression calculations conforming to usual statistical demands. The values are founded on *Benedict-Talbot's* material in 1921.

The regression equation that they found most satisfactory reads as follows:

$$c = 28.12\,w + 8.40\,h - 362.18$$

(c = Cals./24 hrs., w = body weight in kg., h = height in cm.).

By this procedure, the standard deviation around the calculated regression line will also give some idea as to how the compiled material conforms with the actual combination of weight and height. (In the abovementioned equation the standard deviation amounted to 34.34 Calories.) It is possible to devise a simple nomogram, as Levine-Marples did, in order to facilitate the calculation.

In adopting this method, there is always an inherent risk in choosing a rectilinear regression equation, because the minor deviations in the material from the straight line will not be manifest. Such a deviation from the actual conditions seems to occur in this analysis for the older infants.

The explanation may be that the energy metabolism is obtained as a linear function of the weight (3-dimensional) and height (1-dimensional).

Conclusion.

In practically all correlation factors to be noted in the literature, the *body weight* is used in some form or other. When used alone, its exponent is either 1 or ⅔. In some body surface formula the exponent for weight is varying. Still, no factor seems to be decidedly superior to the others. For all these factors, the exponent of the weight has been fixed from the outset, on account of practical or theoretical considerations.

In *Kleiber's* investigations (1931/32, 1943, 1946) we have an instance where the most suitable exponent is produced for the body weight, in relation to the energy metabolism for "individuals" with different body sizes. Although, is does not include any infants, *Kleiber's* investigations, irrespective of the technical procedure, have been of considerable interest and value in the metabolic studies of infants.

For a number of years, Kleiber made comparative studies of the energy metabolism under so-called basal conditions, in different sized adult homeotherms from mice to cattle. He investigated the relation $C = W^b \cdot k$ (C = Cals./24 hrs. W = body weight, k and b constants.) He first plotted the values of calories and weight of the various individuals into a logarithmic coordinate system. When the regression appeared to be manifestly rectilinear, the equation of the regression line ($\log C = b \cdot \log W + \log k$) was calculated. The exponent *b* was found to vary in different combinations from 0.74 to 0.77. *Kleiber* states the exponent at ¾.

Thus, the relationship between energy metabolism and body weight for individual specimens amounted to Cals. $= W^{3/4} \cdot k$.

Moreover, it must be kept in mind that a *combination of two factors* should give a more accurate estimation of a childs size than a single one. (See *Levine-Marples*, 1931.)

Chapter 9

STUDY OF THE RELATIONSHIP BETWEEN THE ENERGY METABOLISM AND THE BODY SIZE

The body weight

In view of the fact that the body weight was used most frequently in order to obtain the relationship between energy metabolism and body size in infants, the body weight has been considered first.

To avoid fixing any special exponent for the weight at the outset, *Kleiber's* procedure has been adopted, viz., to plot values in a logarithmic coordinate system with Cals./24 hrs.[1] along the ordinate, and body weight in kg. along the abscissa. Thus, the relationship between the energy metabolism and the body weight as $C = W^b \cdot k$ will be studied in the logarithmical form

$$\log C = b \cdot \log W + \log k.$$

(C = Cals./24 hrs., W = weight in kg., b and k being constants)

By using this logarithmical form the usual regression formula can be applied.

For the purpose of this study, the author's material and the material found in the literature using comparable methods have been used. In this connection, normal infants have been included, as well as prematures and under-weight children (see Table 6).

[1] The formula Cals./24 hrs. has been adopted (in spite of the statement in page 76, viz., that standard metabolism should be given in Cals./hrs.,) in order to facilitate a comparison between the values recorded in the literature. The conclusious made from mathematical and statistical analyses are not affected by this.

Table 6. *A table showing the number of prematures, normal and underweight infants investigated by various workers, using a method similar to the author's.*

Author[1])	No. of subjects recording weight and height.			No. of subjects recording weight, but not height.		
	Premature	Normal	Underweight	Premature	Normal	Underweight
American:						
Benedict-Talbot	13	70	17	5	2	22
Murlin et al.				15	5	5
Flemming	3		31			4
Levin et al.	15	9	11	16	3	
Clagett-Hathaway		8				
Benjamin-Weech		4				
Total:	31	91	59	36	10	31
German:						
Baer		14	(2)[2])			
Schadow				22	41	
Mordhorst				11	6	
Total:		14	(2)	33	47	
French:						
Fouet				4	10	7
Janet-Bochet				1	72[3])	29[3])
Garot		8	6			
Total:		8	6	5	82	36
Sum:	31	113	67	74	~~99~~ 139	67

[1]) Here each author's figures have been summarised from different publications.
[2]) Two cases delivered prematurely and not examined until 4.0 and 4.4 kg. respectively.
[3]) Ages are not recorded, and some of these children may be more than one year old.

As far as possible, only one value per person has been used for children aged 8 days to 12 months. In materials giving several values for an individual at different age periods, then a value has been chosen in order to give the respective material the most even distribution between different weight groups. For each individual, age and weight, but not always height have been stated, in addition to the caloric value. For this reason, the materials have been divided into two groups, viz. (1) weight and height and (2) weight but not height. In these groups, different materials obtained from the same

author have been combined, although published at different periods (see App. V).

If it was merely a matter of body size, Kleibers comprehensive material for different sized homeotherms could be applied to infants, but the exponent of $W^{\frac{3}{4}}$ cannot be directly applied, because it has been obtained from mature animals and adult material and not from infant material.

Visual inspection of diagrams.

The Author's material has been plotted into Fig. 27. A good correlation is noted. For the smaller infants, the regression appears to be rectilinear, then gradually becoming curvilinear with a deviation towards the weight axis. It will be noted that a similar appearance is obtained when the Cals. are compared with the body weight in kilograms (see Fig. 24, page 83, and Fig. 25, page 85).

The materials from the literature. It is not possible to immediately compare the different materials from the literature because the apparatuses and methods used in connection with the various materials have varied widely from country to country. The materials have been divided into American, German and French (see Figs. 28, 29 and 30).

Comparisons between materials from one and the same country. As far as *normal children* are concerned, the *American* materials appear to be uniform. The *German* materials are rather limited, and have been confined in most instances to a particular age group. *Schadow's* values seem to be somewhat higher than the values in the other German materials. Among the *French* materials, that of *Fouet's* shows a wide dispersion, and this may be easily explained by the method used (see page 16). *Janet-Bochet's* and *Garot's* materials appear to conform to one another fairly well, that of *Garot* possibly lying somewhat lower.

In materials covering both *normal, premature and underweight children,* the prematures seem to follow approximately the same regression line as the normal, though perhaps with somewhat elevated values, while under-weight, full term children, on the other hand, disclose definitely elevated values, when taken together. Among them, various degrees of under-weight are to be found. In the American diagram, it should be noted that *Flemming* (denoted by blue rings) has not investigated any normal material.

A comparison between materials from different countries. As the materials concerning *normal* children, except for Fouet's, seem to be reasonably comparable within the various countries they have been combined nationally and set out in a special diagram (see Fig. 31). In this connection, the prematures have been included, as well as healthy subjects, as previously stated, with a body weight less than 3 kg.

The uniformity between the results obtained from the three countries are good, when one considers their different origins, the different apparatuses employed and the somewhat different investigation conditions.

However, distinct differences are to be noted and therefore one cannot treat this as a homogeneous material.

Regression calculation.

By using regression analysis, an estimate can be obtained of the exponent in the regression equation (page 91) for the weight which during infancy corresponds most adequately to the energy metabolism.

This has been carried out according to the usual formulae (*Snedecor* 1950). The equation for the regression line ($\log C = b \cdot \log W + \log k$) has been determined for the author's material, as well as for the extensive materials from the respective countries.

Notwithstanding the fair uniformity of the diagrams between

the normal materials in each country, the small materials have been excluded.

Among the American materials, those of *Benedict-Talbot* and *Levine* and co-workers have been combined (79 cases), among the French only *Janet-Bochet's* has been chosen (67 cases) and, among the German materials, those of *Mordhorst, Schadow* and *Baer* have been combined, in spite of their difference in age periods, in order to secure a sufficiently large material (62 cases).

The regression equation (in antilogarithmic form), the residual standard deviation[1] and 99 per cent confidence interval for the exponent (b) for the four materials are to be found in Table 7.

All the regression lines from the combined materials of the literature have been superimposed on the author's diagram (see Fig. 27).

When comparing the author's material and the regression lines of others (see Fig. 27), a fair agreement is noted as far as the youngest infants are concerned with a weight less than 6—7 kg. The author's material shows a less marked slope than the regression line for the French material. When the slope is tested, for the author's material and the American material, through a covariance analysis (*Snedicor* 1950), the difference will be found to be significant (see Table 8).

The difference in the materials, which is especially marked with the more advanced children, does not prevent one forming an opinion of the value of the exponent for the weight, in the relationship between the energy metabolism and the body weight during infancy. The exponent values vary between 1.113 and 0.812 with 99 per cent confidence intervals given in Table 7.

[1] By this terms is meant the standard deviation around the regression line, transformed to a percentage proportion. (If the points in the diagram fall exactly on a straight line, the residual standard deviation is zero.) This ~~transformed to a percentage proportion. (If the points in the diagram fall~~ standard deviation of the logarithmic values is 0.0275, the transformed quantity becomes antilog 0.0275 — 1 = 6,6 %.

Table 7. *Results of regression analysis.*

Material	Number of cases	Equation	Residual standard deviation	99 % confidence interval for the exponent b
Author's	60	$C = W^{0.918} \cdot 58.6$	6.6 %	0.844—0.992
Benedict-Talbot + Levine et al.	79	$C = W^{1.000} \cdot 45.71$	13.3 %	0.972—1.208
Janet-Bochet	67	$C = W^{1.113} \cdot 48.4$	12.1 %	1.019—1.207
Baer + Schadow + Mordhorst	62	$C = W^{0.912} \cdot 67.6$	14.8 %	0.753—1.071

Table 8. *Analysis of covariance (logarithmic values). Comparison of the slopes of the regression line between the author's material and Benedict-Talbot + Levine et al.'s material.*

	Sum of squares	Degrees of freedom	Variance	F
Difference between slopes	0.0183	1	0.0183	9.2 ~~8.9~~ ($P < 0.01$)
Distance between regression lines	0.0246	1	0.0246	
Each material around its own regression line	0.2726	135	0.0020	

A comparison with Kleiber's ¾ exponent.

From the width of these intervals it is concluded that the exponent in these regression lines differs significantly ($P < 0.01$) from Kleiber's value of 0.75.

However, the value of *b* tends to fall for older children. Since adults form part of *Kleiber's* material, it would be interesting to estimate the values of the exponent for the age groups between infancy and adolescence. The only material traceable in the literature, that covers the *age periods from birth to adolescence is* that of *Benedict-Talbot* (1921). Benedict-Talbot's entire child material has been plotted into a logarithmic coordinate system, as in the case of the infant materials (see Fig. 32). The regression

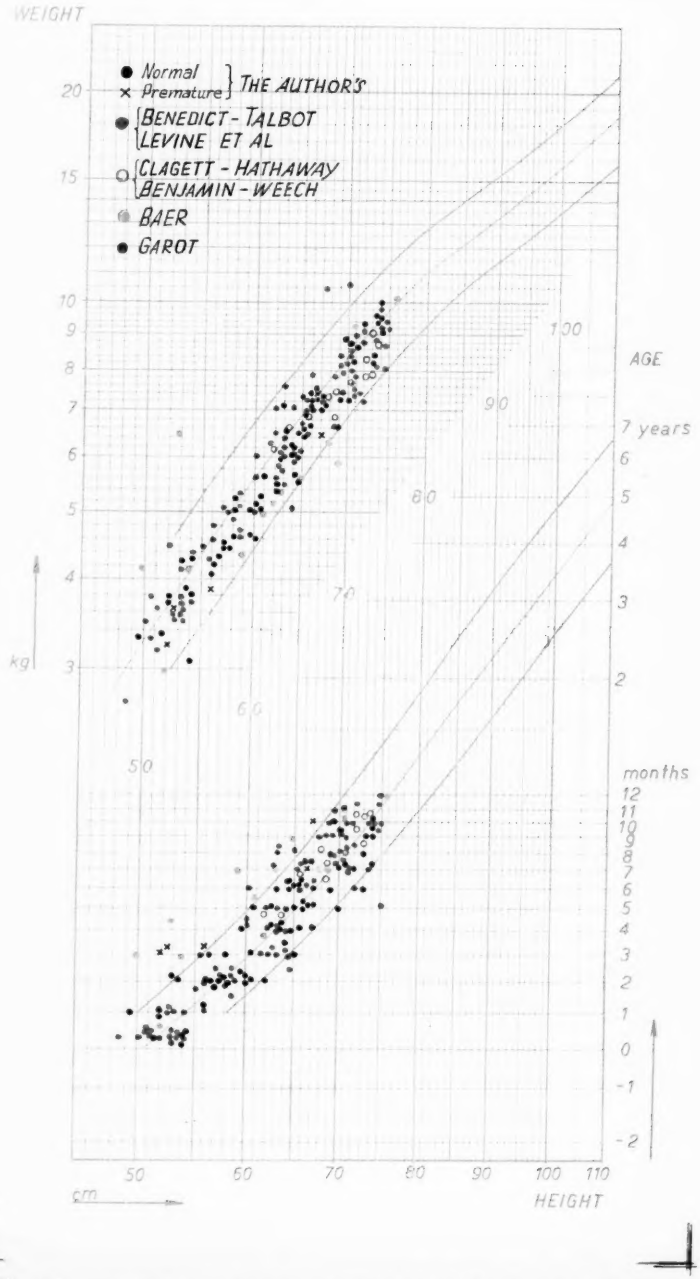


Fig. 26. *A Swedish growth chart. The relationship between the height and the age and between the weight and the height. The 95 per cent confidence intervals are marked. The author's, Benedict-Talbot + Levine et al.'s Clagett-Hathaway + Benjamin-Weech's, Baer's and Garot's normal materials are plotted.*

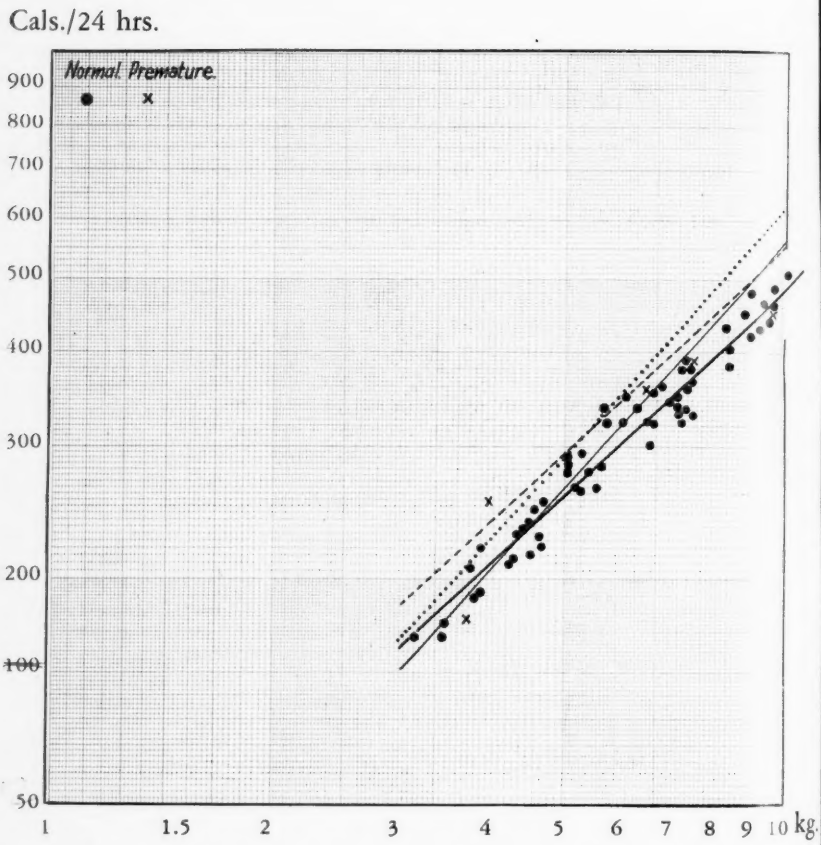


Fig. 27. *The author's material showing the logarithmic relationship between the energy metabolism and the body weight.*

——— *represents the regression line for the author's normal material.*
. . . . . » » » » *Benedict-Talbot + Levine et al.'s normal material.*
. . . . . » » » » *Janet-Bochet's normal material.*
- - - - - - » » » » *Mordhorst + Schadow + Baer's normal material.*

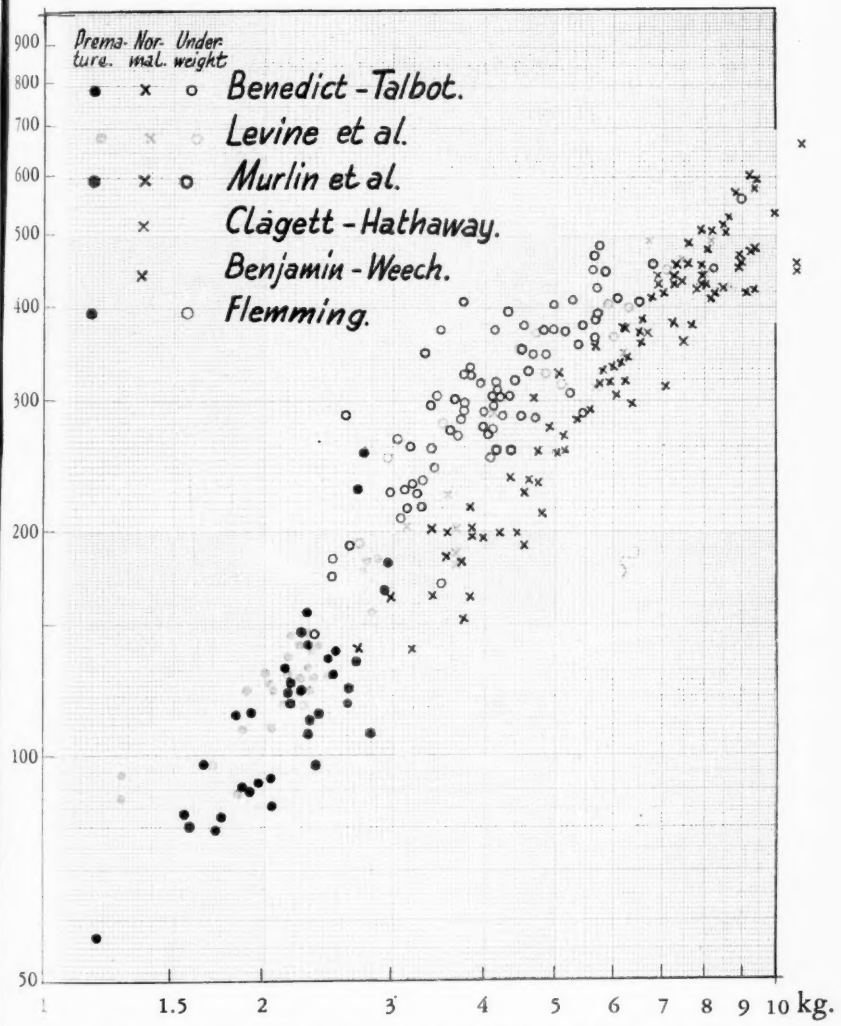


Fig. 28. *American materials showing the logarithmic relationship between the energy metabolism and the body weight.*

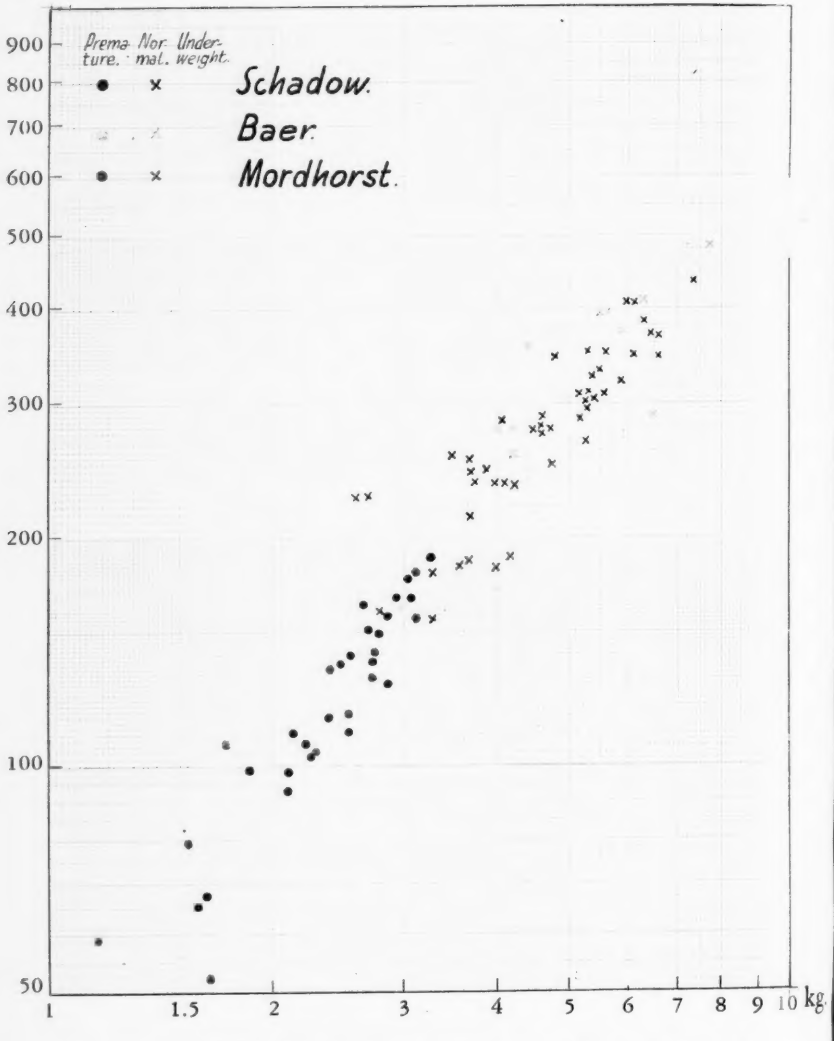


Fig. 29. *German materials showing the logarithmic relationship between the energy metabolism and the body weight.*

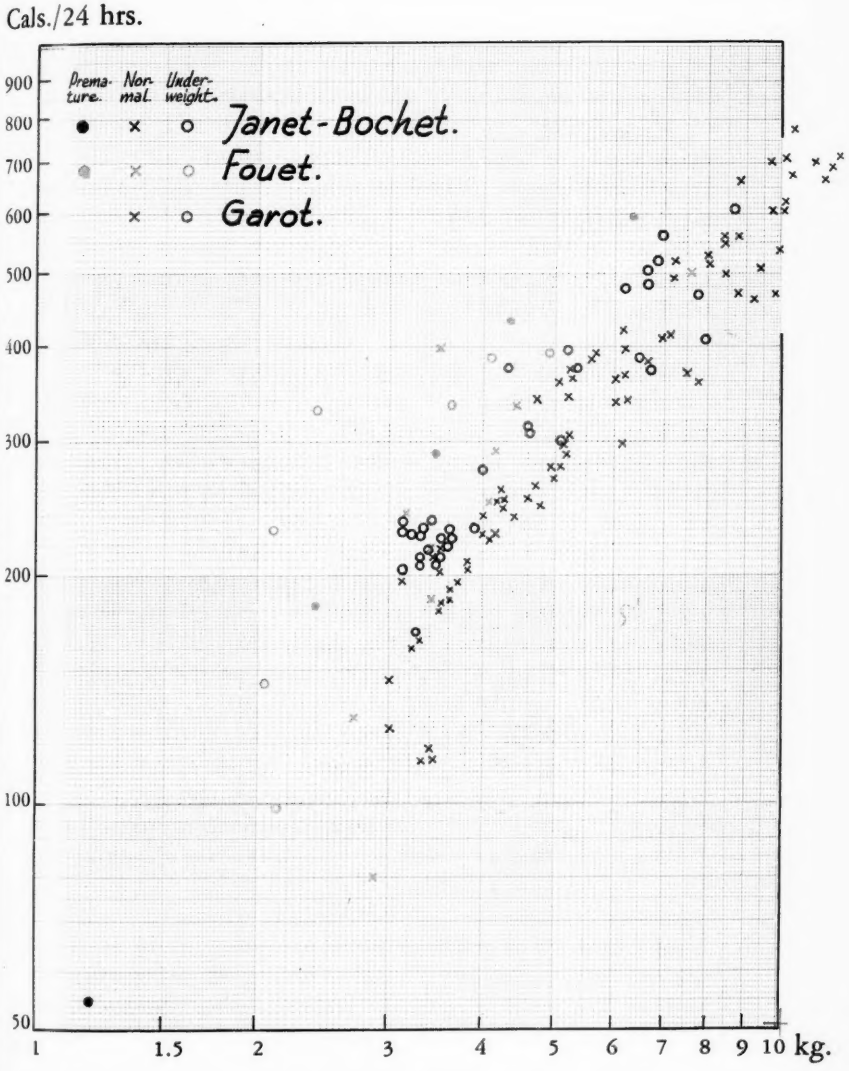


Fig. 30. *French materials showing the logarithmic relationship between the energy metabolism and the body weight.*

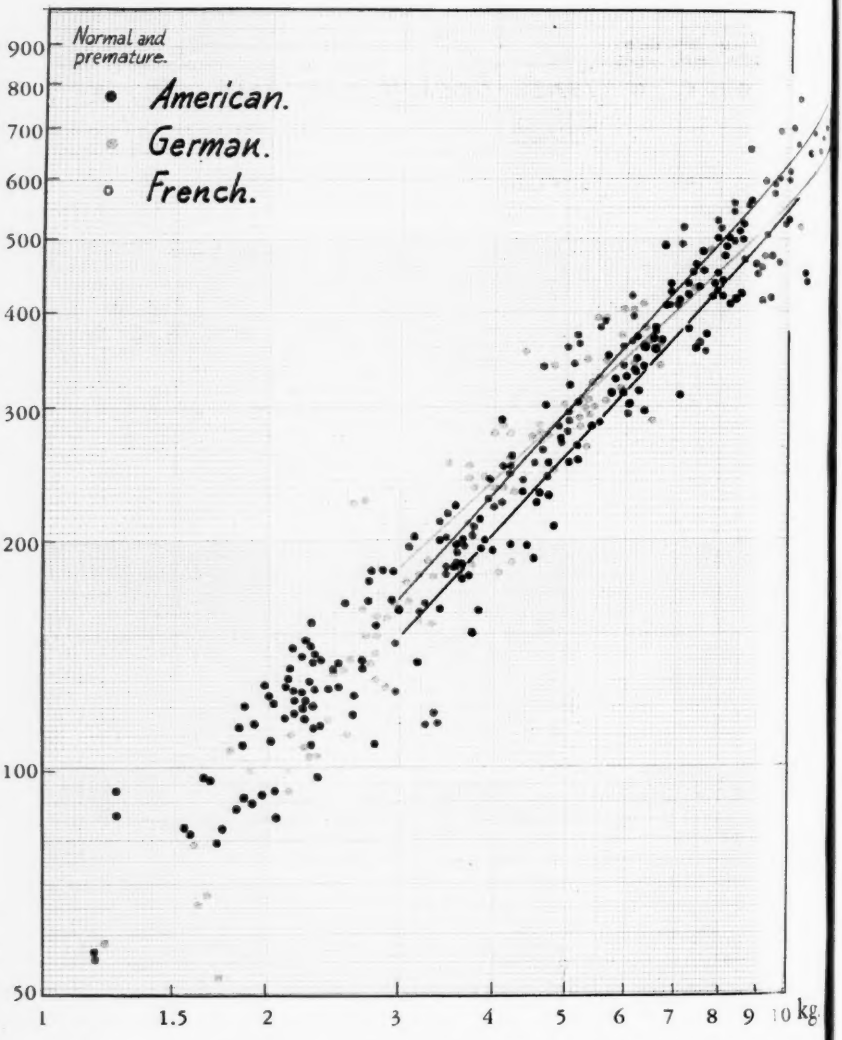


Fig. 31. *Figure summarizing the materials for prematures and normal infants obtained from Figs. 28, 29 and 30. The regression lines are shown.*

line (A) for the author's material and a straight line (B) with a slope of 0.75 (= Kleiber's exponent) have been drawn approximately through the middle of the point values for the older children.

The plotted material reveals a distinct shelf at a body weight of 16 kg. (This will be referred to below). Broadly speaking, the regression line is curved during childhood, but gradually begins to assume a more rectilinear shape towards adolescence. Here, the values group themselves around a rectilinear regression line with a slope of 0,75, i.e. the line that *Kleiber* found applicable to adults and mature animals.

The difference between infants and animals of approximately the same size in *Kleiber's* material is that the infants are at a stage when growth is rapid. The growth is particularly rapid immediately before delivery, and begins to diminish markedly during the first years of life (e.g. *Adams-Poulton* 1937, Weinbach 1941, *Wetzel* 1944). *Wetzel* also points out *that the energy metabolism of a growing individual can be divided roughly into two parts, one serving growth itself and another for maintenance.* That part of the energy metabolism which is necessary for maintenance obviously corresponds to that energy metabolism, which according to Kleiber, is in a relationship to the ¾ power of the weight. When the requirements for the growth are added to this, *b* should be greater than this power. This will partly explain the high value of *b* during infancy. The fact that *b* is reduced already during infancy further emphasizes this supposition, seeing that the growth diminishes likewise.

Discussion.

The weight as a single variable in relation to the energy metabolism, can only be applied to short age periods, because the size of the growth factor is rapidly changing in the case of children. This is noted also during infancy.

When the size of an individual is represented by body weight alone, it is not possible to pay proper attention to the possible variations in the physical proportions of a subject. Variations in

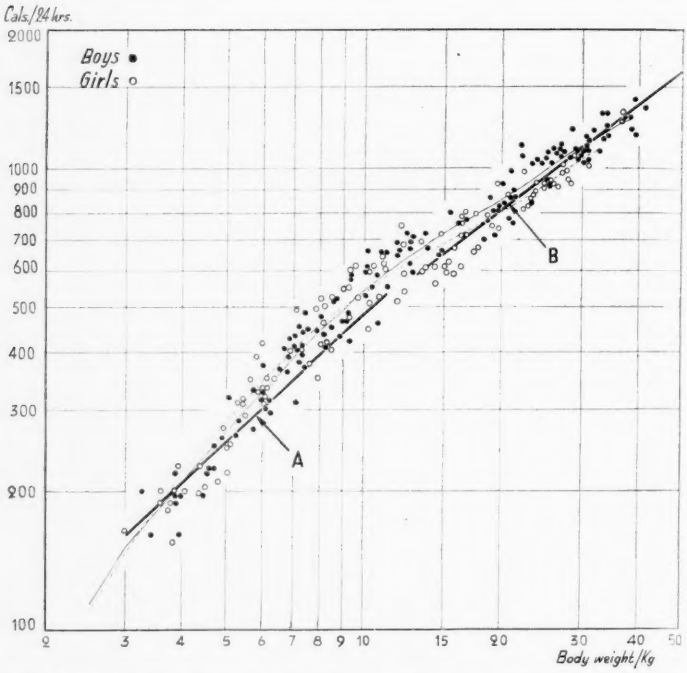


Fig. 32. *Benedict-Talbot's child material from birth to puberty (1921) showing the logarithmic relationship between the energy metabolism and the body weight.*
The regression line for the author's normal infant material (A) and the regression line for Kleiber's material (B) are illustrated in the diagram.

the latter exert an influence on the relationship between energy metabolism and body weight. Every investigator of underweight infants (se App. V) has found a higher value for Cals./kg. for such infants, as compared to normal ones, as will be seen from the Figs. 28, 29, 30. Children of a weight falling 25—35 per cent below the mean weight of their respective age groups have been defined usually as being under-weight. Furthermore, well-covered infants reveal lower value for Cals./kg. since a comparatively larger portion of the mass of the body, as represented by the

weight, consists of metabolically less active adipose tissue. Considering that the physical constitution of infants that otherwise develop normally will vary between relatively wide limits (see the growth diagram in Fig. 26, page 97), *an approximation has to be introduced when the energy metabolism is placed in relation to the body weight alone.*

The body weight and height

When more than one variable is used in order to describe the relationship between energy metabolism and body size, it would seem most natural to choose *weight* and *height*. These body measurements reflect the physical constitution of an individual and his possible deviation from the average.

The author has tried to describe the relationship between the weight and height, on the one hand, and the energy metabolism, on the other, by the expression

$$C = W^b \cdot H^a \cdot k$$

(C = Calories, W = weight, H = height, a, b and k = constants)

By attributing different values to the exponents *b*, and *a*, a variety of different formulae are obtained, and it seems plausible that by suitable selection of the exponents a satisfactory description of the relationship should be obtained. In addition, this formula has the great advantage that, after having taken logarithms, the usual regression formulae can be used:

$$\log C = b \cdot \log W + a \cdot \log H + \log k$$

For this study the author's material has been used together with the materials from the literature, where both weight and height have been recorded (see Table 6, page 92.) Since a combination of weight and height is affected by the bodily structure of an individual, the under-weight children might be included at least theoretically speaking. It should be borne in mind that materials of under-weight children, recorded in the literature, are frequently not fully described. The causes for the under-weight no doubt vary, to a large extent. In addition, prematures have been included.

Visual inspection of two 3-dimensional models.

A 3-dimensional model was constructed at the beginning, with log H and log W for the *x* and *y* axes and log C for the *z* axis, from the author's material and from the materials in the literature.

The author's material revealed a fair correlation, though there was a certain tendency to a faint bend downwards towards the *xy* plane with the older infants.

The materials from the literature although heterogenous show the following tendencies:

1) Among infants with *the same weight*, thin infants have higher caloric value than the normal average sized infant, and this average sized infant has a higher value than the well covered infants.

(2) Among infants with *the same height*, the thin infants have a lower caloric value than the average sized infant, and this in turn is lower than the well nourished infants, but the difference is less pronounced.

(3) The deviation around a supposed regression plane seems to be considerable. In certain materials, such as *Flemmings'*, the caloric values were distinctly higher than in the others, even if allowance is made for the fact that his cases were under-weight. Further the prematures had a position of their own, and they could not fit directly into a more comprehensive scheme.

The materials that seemed to conform to one another were the normal materials of *Benedict-Talbot, Levine* and his co-workers, *Clagett-Hathaway* and *Benjamin-Weech*. The two non-American materials, i.e. *Baer* and *Garot*, were too small to be of value.

In all the abovementioned instances the regression plane showed a slight tendency to bend with the older infants.

Multiple regression calculation.

This tendency of the regression plane to bend, in the author's as well as the materials in the literature, was so slight that it seemed justifiable to submit the materials to a multiple regression analysis.

Table 9. *Results of multiple regression analysis.*

	Regression equation	Residual standard deviation log	Residual standard deviation transformed in per cent	95 % confidence interval log	95 % confidence interval transformed in per cent
hor's material:					
mber of cases = 60					
s./weight	$C = W^{0.918} \cdot 58.6$	0.0279	6.6	0.0558	+ 13.7 to — 12.1
s./height	$C = W^{2.538} \cdot 0.00832$	0.0393	9.5	0.0786	+ 19.8 to — 16.6
s./weight and height	$C = W^{0.611} \cdot H^{0.951} \cdot 1.99$	0.0215	5.1	0.0430	+ 10.7 to — 9.4
edict-Talbot + ine et al.'s material					
mber of cases = 79					
s./weight	$C = W^{1.090} \cdot 45.71$	0.0543	13.3	0.1086	+ 28.4 to — 22.1
s./height	$C = W^{2.917} \cdot 0.00189$	0.0482	11.7	0.0964	+ 24.1 to — 19.9
s./weight and height	$C = W^{0.387} \cdot H^{1.936} \cdot 0.0541$	0.0459	11.2	0.0918	+ 23.5 to — 19.0

Among the various materials in the literature, only *Benedict-Talbot's* and *Levine* et al.'s combined material for normal individuals can be considered, because the others are too small. The materials of the under-weight subjects can not be employed, because they are too unevenly distributed between the different age groups.

The antilogarithmic equations for the regression planes, as well as for the regression lines with calories to weight only or to height only will be found in Table 9. Further, the residual standard deviations[1] and 95 per cent confidence intervals have been computed for each equation and are given in the table in logarithmic and antilogarithmic form.

This procedure diminished the residual standard deviation for *the author's material* from 6.6 per cent to 5.1 per cent, when body weight alone was used. This difference, which is significant ($P < 0.01$), will not appear particularly large. However it transforms a 95 per cent confidence interval for the variation round a standard value from + 10.7 to — 9.4 per cent, instead of

[1] See note page 95.

Table 10. *Analysis of covariance (logarithmic values).*

	Sum of squares	Degrees of freedom	Variance	F
Comparison of the slopes of the regression plane between the author's material and Benedict-Talbot + Levine et al.'s material.				
Difference between slopes	0.0178	2	0.0089	6.4 ($P < 0.01$)
Distance between regression planes	0.0347	1	0.0347	
Each material around its own regression plane	0.1864	133	0.0014	
Comparison between one-factor and two-factors. Author's material: weight-factor and weight-height-factor.				
Decrease of variation when height is included	0.01891	1	0.01891	41 ($P < 0.01$)
Variation around the regression plane	0.02638	57	0.00046	
Variation around the regression line (Cals./weight)	0.04529	58		
Benedict-Talbot + Levine et al.'s material: height-factor and weight-height factor.				
Decrease of variation when weight is included	0.01877	1	0.01877	8.9 ($P < 0.01$)
Variation around the regression plane	0.16005	76	0.00211	
Variation around the regression line (Cals./height)	0.17882	77		

+ 13.7 to — 12.1 per cent. This, no doubt, has a practical significance. See Table 10.

As far as *Benedict-Talbot's* and *Levine* et al.'s combined material is concerned, which is the only comparable comprehensive

material that is available in the literature, the difference in the residual standard deviation is smaller, but it is significant. The explanation is, that the residual standard deviation is more than twice the size. See Table 10.

The "capacitance surface"

The calculations would be simplified if the weight and height of the body could be combined into one single factor which would represent the size of the body at different ages. If the regression between this factor and the energy metabolism is rectilinear then one would avoid an approximate result for the older infants.

Such an attempt has been made by using the electrical capacitance of the body surface of the child to represent its body size.

Method of determination (elaborated by *Karlberg-Lind* ~~1947~~, ~~1951~~).

According to this method, the child is placed on an isolated frame in the middle of a room, in which the ceiling, walls and floor are covered with earthed netting wire. The arms and legs are extended and held in a position, at about 45 degrees to the body axis.

The child's body surface area and the wire netting form a condenser, whose capacitance is dependent on the size of the surfaces. This capacitance only varies with the size of the body surface of the child when determinations are made in the same room. The capacitance is determined by an apparatus which is placed in a corner and it represents the difference in adjustment of the resonance in a circuit in a variable condenser, when the child is connected, and when it is not connected in the circuit. A weak, high frequency alternating current (500.000 cycles per second) is used. The examination takes about five minutes, and is simple and can be managed by a nurse.

In order to be able to transfer the capacitance value to a

surface measurement, irrespective of the size of the room space, the latter was calibrated by measuring the capacity of a number of spheres of varying size with electrically conducting surfaces. The surface area of these spheres is easily found geometrically and the relationship between the electrical capacitance and the surface area is obtained from bodies with a spherical form. By means of this relationship between the capacitance and the surface area the capacitance of the child was transferred to a surface measurement, which is independent of the size of the room.

However, the capacitance is dependent not only on the surface area of a body but also on its shape, i. e., a more spherical surface gives, per surface unit, a smaller capacitance than an elongated surface. Accordingly, the calculated body surface area of a child is greater than the actual surface but it gives a relative value of the body surface area in surface units. Since the difference in the shape of the body also influences the electrical capacitance then the surface determined from the capacitance (the "capacitance surface") will give a body measurement which is dependent upon the body size.

The direct determination of the "capacitance surface" of an individual can be rapidly performed, but the apparatus is rather expensive and requires a lot of space, and is therefore unsuitable from a practical point of view.

Calculation of the "capacitance surface" from weight. and height.

The capacitance surface was determined on 130 infants differing in age and physical proportions. The relationship between these values to the actual weight and height of the individuals was studied. They formed the basis for *a nomogram.*

For the construction of this nomogram, the height-weight part of the growth chart is used. The mean value curve for the weight-height, which is a slightly curved line was graduated in units for the "capacitance surface," by proceeding from the recorded values in the investigated material.

The relationship between individuals with the same "capacitance surface," but differing in physical structure has been approximated to a straight line in this logarithmic coordinate system, in accordance with the procedure previously applied to the multiple regression calculations. The angle between these lines and the mean curve, i.e. "the same capacitance surface lines" has been tested over short portions of the mean line and the angle is found to be about 100 degrees. A 3-axial nomogram has been constructed from the graduated mean curve and the 100 degrees angle between the mean curve and the "same capacitance surface lines" (the construction will be seen from Fig. 36. page 120).

The relationship between the energy metabolism and the "capacitance surface".

The "capacitance surface" for individuals in the metabolic material of the author has been calculated from the abovementioned nomogram.

The body size of the subjects is represented now by only one variable and thus the relationship between the standard energy metabolism and the body size can bee illustrated by plotting the values in a 2-axial diagram. Fig. 33 shows the author's material. In this nonlogarithmical diagram the regression seems to be rectilinear. An ordinary regression analysis, gives the equation for the normal material, i.e.

$$C = 995 \cdot S - 52$$

(C = Cals./24 hrs., S = "capacitance surface" in sq.mt) with a residual standard deviation of $\pm$ 21.8 Cals.

A direct comparison between the residual standard deviation from this analysis and the multiple regression cannot be made, since the latter is obtained from a percentage value. None the less, the "capacitance surface" should be preferable, especially with regard to the more developed children.

Conclusion.

With regard to the relationship between the energy metabolism and the body size, it is not sufficient to rely on body

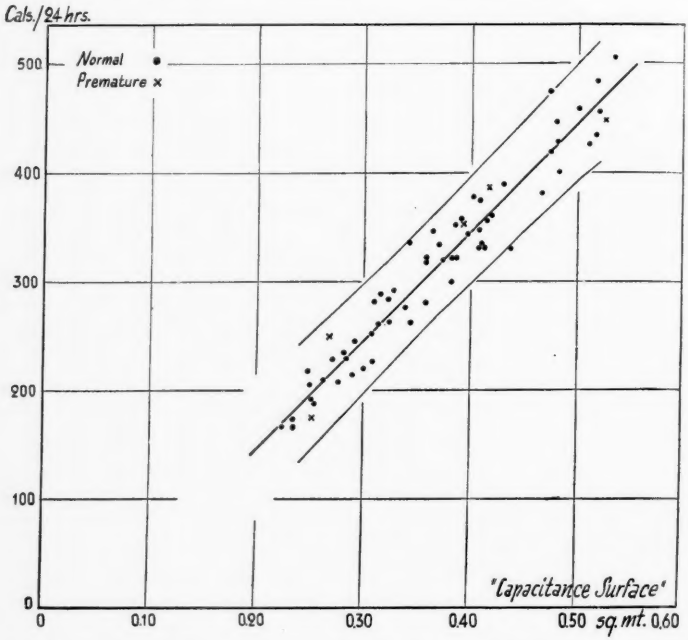


Fig. 53. *The author's material showing the relationship between the energy metabolism and the "capacitance surface". The regression line with the 95 per cent confidence interval is shown.*

weight alone as representing the body size. When a combination of body weight and body height is applied, as in the relationship $C = W^b \cdot H^a \cdot k$, or as in the calculation of the "capacitance surface," then an analysis of the author's material in the present investigation shows a residual standard deviation round the regression of about ± 5 to 7 per cent for infants with a body weight of 6—7 kg. Since the random error of the present method for an analysis of the standard metabolism, i.e. the variation for an individual in an investigation, remains around 3—4 per cent, the reliability of these two correlation procedures must be accepted as sufficient for routine clinical practice.

Chapter 10

STANDARD ENERGY METABOLISM IN INFANTS BETWEEN ONE WEEK OLD AND ONE YEAR

Normal values from the literature

Rubner's law (1893). The values for energy metabolism in infants derived from *Rubner's* law must be regarded as the first recorded for normal infants. The energy metabolism would, according to this law, be proportional to the body surface of an individual, calculated according to *Meeh's* formula. A mean value of 1,000 Cals./24 hrs. was said to be generally applicable. However, it has been found not to be valid for infants (see the analysis of correlation factors page 87. Moreover, it was designed for use in tests extending over several days, which is an additional reason for repudiating it.

Benedict-Talbot's normal values (1921). In their monograph, entitled "The metabolism from birth to puberty", Benedict-Talbot have included a table of normal values for "basal heat production of boys and girls per 24 hours predicted from body weight". The values were obtained from their large child material and the Cals./24 hrs. were plotted against the body weight and a mean curve has been inserted, smoothed by inspection (for the boys, see Fig. 25, page 85. In the table, boys and girls are presented separately. The caloric value is stated for 2.5 kg., and then for each additional ½ kg. up to puberty. The difference between the sexes during infancy is insignificant. The table requires interpolation. Benedict-Talbot's material has been more satisfactorily analysed by *Levine-Marples.*

Kestner-Knipping's standard tables (1924). The correlation between energy metabolism and body size represented by weight and age, as used in the abovementioned tables does not seem to have been adequately analysed (see the analysis of correlation factors page 89). The normal material, on which it is based, has not been accounted for. The tables contain wide intervals. Consequently, a laborious interpolation is necessary in most cases. Nevertheless, their standard values have been employed by several German scientists, e.g. by *Beck, Baer, Schadow, Mordhorst*. They consider that they have obtained a good conformance with direct determinations made on normal infants from these standard values.

Levine-Marples' normal values (1931). They are founded on a multiple regression calculation made on *Benedict-Talbot's* normal material at an age of 8 days—25 months. 137 values have been analysed, viz., 73 values from 40 boys, 64 values from 33 girls. The regression equation considered by them to be most practical was as follows:

$$c = 28.12 \cdot w + 8.40 \cdot h - 362.18$$

(c = Cals./24 hrs., w = body weight in kg.,
h = height in cm.)

They found no significant difference between the sexes. It should be noted that if values for an individual are recorded on more than one occasion in the material, then the usual regression formula cannot be applied in general when all the values have been combined to give a single material. This is the reason why the author in his analysis of the literature has taken only one reading from each individual (page 92).

No other direct normal values for energy metabolism during infancy have been traceable in the literature. Several other authors have studied the energy metabolism in normal infants, presenting original values, either in the form of tables or as plotted diagrams, though without stating any direct normal values or normal variations.

Conclusion. None of the abovementioned normal values appears to be directly applicable to infants. Those founded on *Rubner's* law have not been used. *Benedict-Talbot's* normal values have been replaced by those of *Levine-Marples*. *Kestner-Knipping's* normal values must be regarded as doubtful. The values of *Levine-Marples* alone remain acceptable, although the statistical analysis does not seem to be quite satisfactory.

Values from the author's material

In accordance with what has been said in Chapter 9, the values in the author's normal material for standard energy metabolism have been related to the individuals body size, which is obtained from the body weight and height. These two body measurements have been used either as two variables in a multiple regression equation, or for calculating the "capacitance surface".

The relationship between the energy metabolism and the body size has been found to be

$$C = W^{0.611} \cdot H^{0.951} \cdot 0.0829 \text{ (see page 107) or}$$

$$C = 955 \cdot S^{1.215} - 2.17 \quad \text{(see page 111)}$$

(C = Cals./hr., W = weight in kg., H = height in cm., S = "capacitance surface" in sq.mt.)

transformed in Cals./hr., with a residual standard deviation of 5.1 per cent and 0.91 Cals./hr. respectively.

A comparison with the values in the literature.

Comparable normal material obtained from the literature is given in Table 6 (page 92). Unfortunately in about 50 per cent of the material, the body size of the individuals has been represented by the body weight alone. Therefore to get as close a comparison as possible, between the author's values and the values in the literature the relationship between the energy metabolism and the body weight has been compared. A graphical comparison is shown in Fig. 27—31 (page 98—102) and the more extensive materials have been compared statistically, see Table 7 and 8 (page 96). A difference has been found which is of statistical significance.

A dissimilarity in the physical constitution of the individuals in the different materials may explain this difference, when one considers that an under-weight infant produces a higher caloric value per kg. of body weight. It is only possible to form an opinion on such differences, when both the height as well as the weight have been given. This can be done quite simply by plotting the various individual values into the growth chart, see Fig. 26 (page 97).

A visual inspection of the chart reveals a distinct conformance between the author's material and the American material. The French material *(Garot)* is too small (6 cases). This French material and the German material by *Baer* show a bigger deviation from the average.

Thus, the American material is the only material that can be compared with the author's material, with regard to weight and height (i. e. the combined material of *Benedict-Talbot* and *Levine* et al.'s).

For this purpose, a comparison is made between the multiple regression equations for the two materials, see Table 9 and 10 (page 107 and 108). A statistical significant difference is shown, between the slopes of the two regression planes.

In order to show this difference graphically, the "capacitance surface" has been calculated from the body weight and height for the American materials, and the regression line for the relationship between the energy metabolism and the "capacitance surface" has been computed. Fig. 34 shows the regression lines for the two materials.

In order to compare two regression equations it is necessary to estimate the degree of the uncertainty of the points on the regression lines. This estimation can be made as follows. Let x_1 be a given value of the "capacitance surface". A 95 per cent confidence interval for the Cals. corresponding to x_1 can be calculated from the formula

$$C \pm 2 \cdot s \sqrt{\frac{1}{n} + \frac{(x_1 - \bar{x})^2}{(S)}}$$

where *s* is the residual standard deviation, *n* the number of cases and *(S)* the sum of squares of the x-values around their mean.

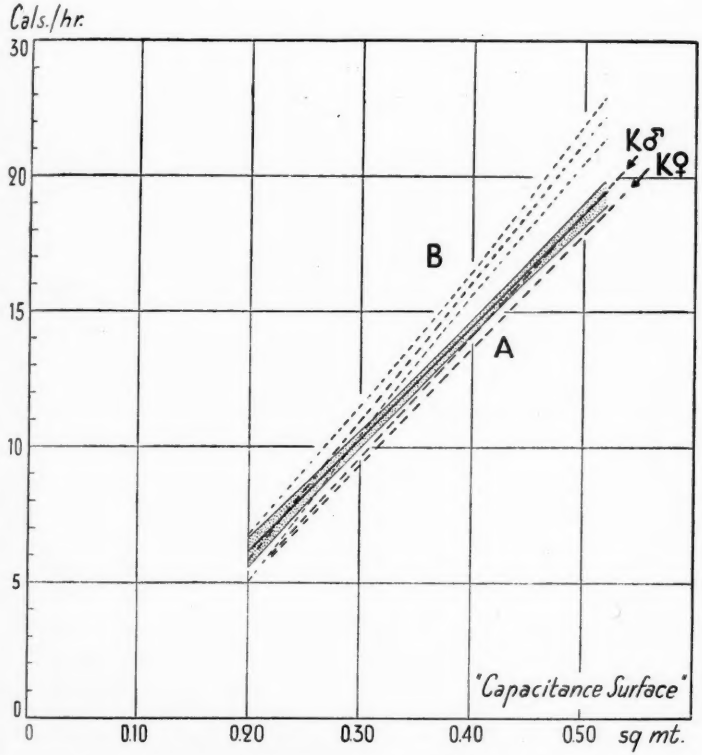


Fig. 34. *The relationship between the energy metabolism and the "capacitance surface" for the author's material and for Benedict-Talbot + Levine et al.'s material. The regression lines with their sampling error are shown, (A) and (B) respectively. Calculated values from Kestner-Knipping's standard tables for infants with an average body constitution at different ages are connected by a line (K). The sexes are dealt with separately.*

In Fig. 34 two curves have been drawn through the end-points of these intervals for each material.

As several German authors have found the Kestner-Knipping's standard tables very useful, the caloric values and the "capacitance surface" for infants with an average body constitution

have been calculated for different ages and sex. These values have been plotted in the diagram in Fig. 34 and connected with a curve for each sex.

Fig. 34 shows a good uniformity between the author's material and the American material for the smaller infants, but as the infants become bigger a difference appears in which the American values are higher. At an age of 1 year, the difference is about 12 per cent. In comparison with *Kestner-Knippings* values the author's material shows higher values for the smaller infants, but about the same for the bigger infants.

Discussion.

When one examines the abovementioned growth chart it becomes clear that the individuals in the author's and the American materials have a uniform physical constitution. The apparatuses used to determine the respiratory exchange can be regarded as comparable. The alcohol test produces similar results.

In the author's material, the calculation of the energy metabolism has been based on the oxygen consumption, whilst the greater part of the American material is derived from the production of carbon dioxide, but this should not make any difference.

However the investigation conditions differ. In the present material, the investigations have been performed in the morning, or early forenoon whilst fasting. The American investigators have, as a rule, made their investigations during the day, after a small meal has been administered.

It is an established fact that food increases the energy metabolism the first hours after a meal. The influence is less marked in young individuals than adults.

According to the author's and *Å. Gyllenswärd's* investigations, a 24 hour rhythm for the energy metabolism appears to be present. For this reason, the time during which the investigations have taken place is not without significance. Furthermore, there is reason to suppose that the energy metabolism is lower in the morning than later in the day (see Chapter 3).

If the investigation conditions for the American infant material

are compared with those for the fasting older children in the whole Benedict-Talbot's child material, it will be seen that they give rise to an increase in the energy metabolism. This will be seen in Fig. 32, page 104, where this material from birth to puberty has been plotted into a logarithmic diagram for Calories and body weight.

Precisely at the body size at which there is reason to assume that the investigation conditions of the children have been modified, a distinct shelflike change is visible.

The regression line of the author's material has been inserted into this diagram. It is seen to conform more evenly with American material for the older children.

In the French material, *Janet-Bochet's* investigation conditions were similar to those used in the American infant material.

The difference in the energy metabolism of the older infants in the author's material and the American material can be explained by the differences in the investigation conditions.

The difference between the younger infants and *Kestner-Knipping's* standard values is more difficult to judge, since no original values for the energy metabolism have been given and there is no data regarding their investigation conditions.

Conclusion.

Except for certain differences in the investigation conditions, the author's values for the standard energy metabolism apparently conform to values obtained from otherwise comparable metabolism investigations on infants.

The values obtained from the author's normal material have been used to form two nomograms for predicting the standard energy metabolism for infants from 1 week old to 1 year old. One based on the multiple regression equation, the other on the "capacitance surface," see Fig. 35 and 36 and in both the body size is represented by the weight and height.

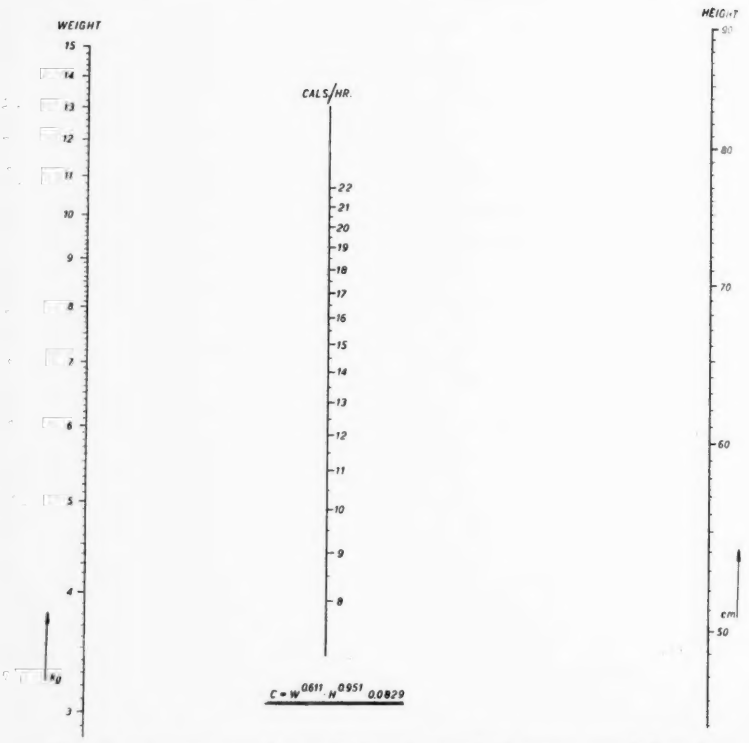


Fig. 35. *A nomogram for predicting the standard energy metabolism from the weight and height according to the multiple regression equation.*

Application of the method

In order to obtain some preliminary ideas on the practical value of the method a number of infants with undoubted hypothyroidism have been examined before and during treatment. The results are given in App. IV. The values obtained have been put in relationship to the "capacitance surface" and plotted in a diagram, see Fig. 37. In this diagram also the 95 per cent confidence intervals around the regression line are given. These are calculated from the sum of the residual standard deviation

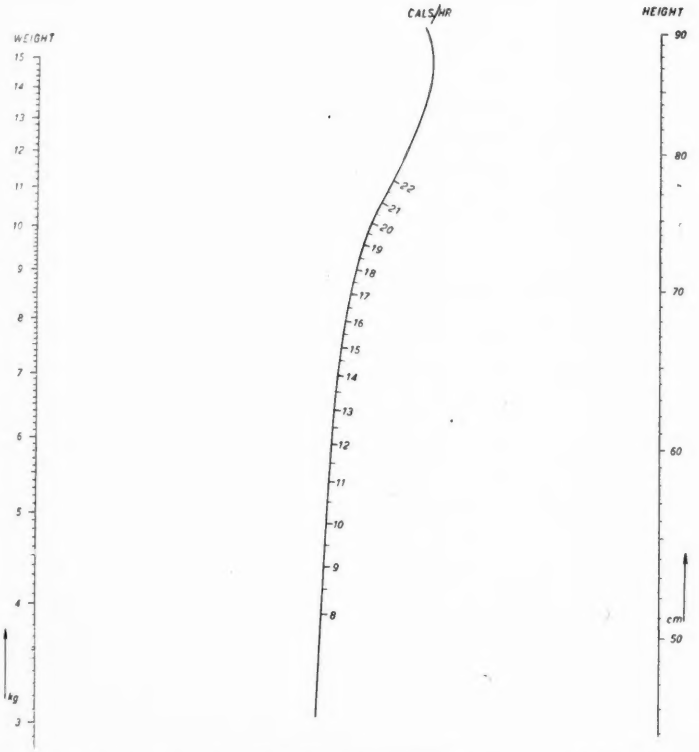


Fig. 36. *A nomogram for predicting the standard energy metabolism from the weight and height according to the relationship between the standard energy metabolism and the "capacitance surface".*

around the regression line, and ~~from~~ the ~~residual standard deviation~~ of the line itself.

As is seen from this figure, the infants have values clearly below the 95 per cent confidence interval before treatment. During treatment their values are within the 95 per cent confidence intervals.

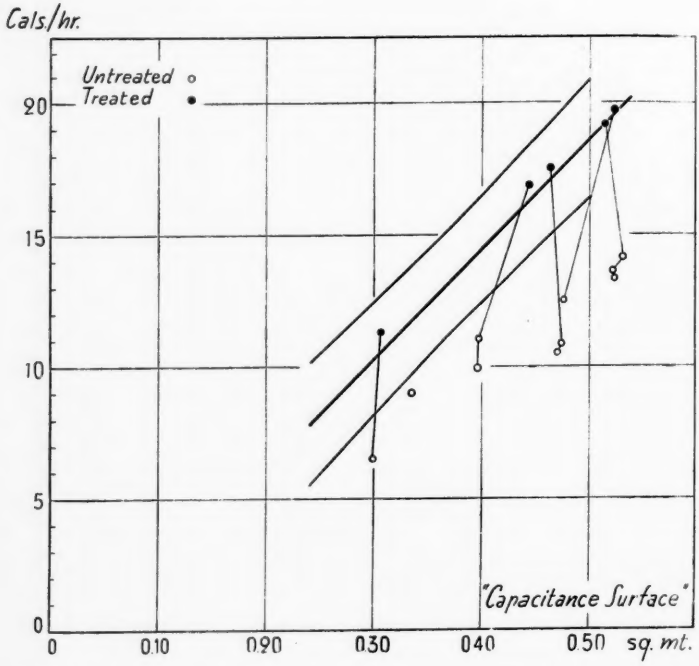


Fig. 37. *Comparison between untreated and treated cases of hypothyroidism and the author's normal material which is represented by the regression line with 95 per cent confidence intervals.*

Chapter 11

AN OPEN CIRCUIT METHOD, SPECIALLY DESIGNED FOR THE NEWBORN

In a study of energy metabolism in infants, immediately after birth, due regard must be paid to the special conditions prevailing at that period of life. As previously stated, earlier investigations, and particularly those of *Benedict-Talbot* (1915), *Murlin* et al. (1925), as well as *Marine* et al. (1922), have shown that the energy metabolism is lower during the first week of life than later, and that the respiratory quotient during the first week appears to vary in a systematic way. Immediately after delivery the respiratory quotient is almost 1 according to *Hasselbalch's* investigations (1904). It then falls, during the next few days to 0.70, but rises again, after a further lapse of a few days, up to about 0.86, i.e. the general mean value for infants. This implies, that in studies of the energy metabolism of newborn, both oxygen consumption and the production of carbon dioxide should be determined and at the same time amounts measured should be small. For such a purpose, an apparatus based on the principle of the open system is most suitable (see descriptions of apparatuses in Chapter 1). To this has to be added that after delivery, according to *Hasselbalch's* observations, the changes in the respiratory exchange are so rapid and therefore the observation periods have to be short. Accordingly, the respiration chamber must be as small as possible in order to shorten the preliminary period, before the actual observation period.

Malm's method (see p. 30), viz., to apply a small rubber mask to the face

of the subject, and then measure the respiratory exchange according to the Douglas bag principle, appears to be a radical solution. It presupposes, that masks are available to fit different sized faces. Moreover this procedure must trouble a great many infants and it will be difficult to quieten some of these subjects.

As a consequence of the abovementioned observations, the following method has been devised.

The present method.

Respiration chamber. In order to cause the child as little disturbance as possible and still keep the respiration chamber small, a plastic hood[1] has been used, which is open at the bottom, and one of the walls consists of a soft plastic material. The hood is big enough to pass easily over the head of the infant (see Fig. 38 and Fig. 40). A piece has been removed from the soft plastic material in order to provide for the neck of the child. The part of the head of the bed is smoothed out.

A fan type of pump sucks fresh air through a tube at the upper end of the wall opposite the infants mouth into the hood, and the air passes out by way of a tube at the opposite end and, finally from here via a mixing vessel and a water vapour filter out through a gasometer. The respiration chamber is not completely hermetically sealed, but owing to the fact that the tube for the intake of air is wide, and also the air is sucked through the chamber, thus producing a slight negative pressure inside, none of the respiration products of the subject is lost.

The gasometer following careful calibration shows an error less than 0.5 per cent.

The analysis of the outgoing gas is taking place continuously during the course of the investigation. A small part of the gas mixture passing from the mixing vessel (about 0.1 lit./min.) is sucked through a so-called "*Gaswechselschreiber*," as described by *Rein* (1925).

[1] The same hood with a slight modification is used for penicillin aerosol treatment in infants (*Karlberg, Sterner* and *Wallmark* 1950).

Fig. 38. *Photograph showing the open circuit method.*
1. *Analysor of the "Gaswechselschreiber".*
2. *Galvanometers.*
3. *Camera.*
4. *Pumps.*
7. *Time recorder.*
8. *Plastic hood.*
9. *Control gas cylinder.*
10. *Tube for incoming air.*

Rein's "Gaswechselschreiber". The principle is to measure the heat discharge from a heater wire to a gas flowing at a constant rate. The capacity of the gas to absorb heat, which is dependent on the composition of the gas, is measured by the diminished resistance in the heater wire, caused by the cooling. The gas that is to be analysed is compared with a gas of known composition and the heat-absorbing capacity is measured in the same way. Since it is the difference in the concentrations that is wanted, the difference in the heat-absorbing capacity of the flowing gases is measured directly. The two gases are made to flow simultaneously through two tubes equipped with a heater wire, and the velocity and other conditions are identical. The difference in the resistance of the two heater wires is measured, according to *Wheatstone's* bridge procedure.

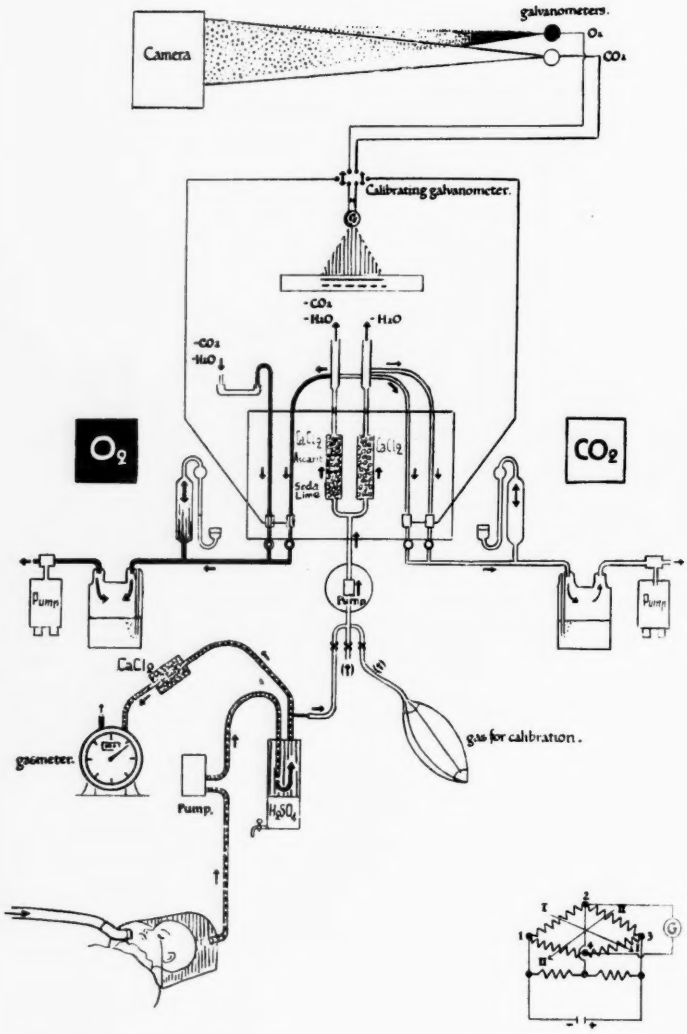


Fig. 39. *Diagramatic representation of the open circuit method.*

The gas which has to be analysed for oxygen and carbon dioxide is dealt with in the following way: By means of a membrane pump the gas is driven into the bottom of a U-shaped tube, and it is thus divided into two, and the gas in both the tubes is made to pass through a water vapour filter. In one of them it passes through a carbon dioxide filter as well. The difference in heat-absorbing capacity of the gases in the two tubes is determined. The gases are identical except for their carbon dioxide content. The gas from which the carbon dioxide is removed is compared also with fresh air after first removing the water vapour and carbon dioxide. The difference in changes in the resistance of each pair of the heater wires is measured from the deflections on the galvanometer in the respective *Wheatstone* bridges. The deflections of these two galvanometers are recorded on a running band of sensitive photographic paper. Because of the fact that a change in the concentration of the gas undergoing analysis produces a rapid change in the galvanometric deflections, the changes in the concentrations will be recorded by continuous registration. The galvanometric deflections, resulting from the analysis, are checked with deflections obtained from a control gas mixture with a known carbon dioxide and oxygen content, made before or after the actual analysis (in a rectilinear relationship). This control gas, which is preserved in a gas cylinder, and is sufficient for a number of analyses, is analysed according to *Haldane's* technique. The mechanism of the gas exchange recorder will be seen from the diagram in Fig. 39.

The gas exchange recorder analyses and registers continuously the air leaving the respiration chamber. It gives the carbon dioxide concentration and the difference in oxygen concentration between fresh air and the gas, after carbon dioxide has been absorbed.

In order to analyse the control gas as carefully as possible *Enghoff's* modification of Haldane's technique has been used.

Investigation procedure.

The plastic hood is placed over the head of the subject.

During the investigation, the child occupies a recumbent position in bed.

At the head end, the bed is smoothed out, before the plastic hood is applied. As an extra tightning precaution, a piece of very soft plastic material is placed over the neck of the child. The hood is ligthly pressed downwards. The pump is started and it drives fresh air through the hood to the mixing vessel, humidity filter and the gasometer. The gas exchange recorder is coupled to

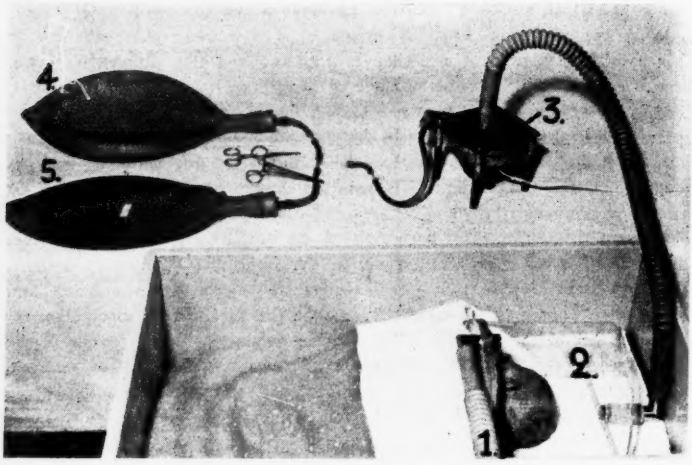


Fig. 40. *Photograph showing modification used in the method.*
1. *Tube for incoming air.*
2. *Plastic hood.*
3. *Pump.*
4. *Filling bag.*
5. *Empty.*

the mixing vessel. The pump is adjusted to a capacity that will produce a difference in concentration between ingoing and outgoing air of about 0.5 vol. % for the oxygen and the carbon dioxide. The difference in concentration is taken directly from the gas exchange recorder. A stop-watch is started and the gasometer read. After a few minutes gas equilibrium is obtained in the hood. The investigation is continued for 10—20 minutes. There should be at least a 10-minute-observation-period as soon as the child becomes still. Movements by the subject can be registered by a light signal, which is recorded on the record paper. These movements are therefore clearly visible when one comes to analyse the record for the changed concentrations. The gasometer is read every 5 minutes. At the end of an investigation the temperature in the gasometer is noted. Then the control gas is

driven through the gas exchange recorder, and the barometric pressure is measured. A typical record see Fig. 41.

A modification of the method.

If a child cannot be taken to the laboratory, the air pumped from the hood can be transferred to rubber bags, which can hold 8—10 litres each. Each bag is filled in 2 minutes. During the first 2 minutes no gas is collected, since it takes at least 2 minutes to obtain gas equilibrium. 5—6 bags will be sufficient provided the child has kept quiet all the time (see Fig. 40). The bags are then analysed by means of the gas exchange recorder. Their volumes are measured by means of the gasometer. A typical record see Fig. 41.

Calculation.

Definitions:	Volume	Concentration of O_2	CO_2	N_2
Ingoing air	V_I	21%	0	79%
Outgoing air	V_E	O_E	CO_2	N_E
after CO_2 absorption		O_A		N_A
Determinations:				
Gas exchange recorder		$21 - O_A = O_x$	CO_2	
Gasometer + 0.10 lits./mt. (corrected for barometric pressure and temp.)	V_E			

Calculations:

Oxygen consump. $= \frac{V_I \cdot 21}{100} - \frac{V_E \cdot O_E}{100} = \frac{V_E (100 - CO_2) \cdot O_x}{100 \cdot 79}$

$$\left(V_I = \frac{V_E \cdot N_E}{79} ; N_E = \frac{N_A (100 - CO_2)}{100} ; N_A = 100 - O_A \right)$$

Carbon dioxid produced $= \frac{V_E \cdot CO_2}{100}$

Respiratory quotient $= \frac{79 \cdot CO_2}{(100 - CO_2) \cdot O_x}$

The energy metabolism is obtained from the amount of oxygen consumed. The caloric value for 1 litre of oxygen is obtained from the respiratory quotient in *Michaelis'* nomogram (Fig. 21, page 69) with a supposed protein metabolism of 15 per cent.

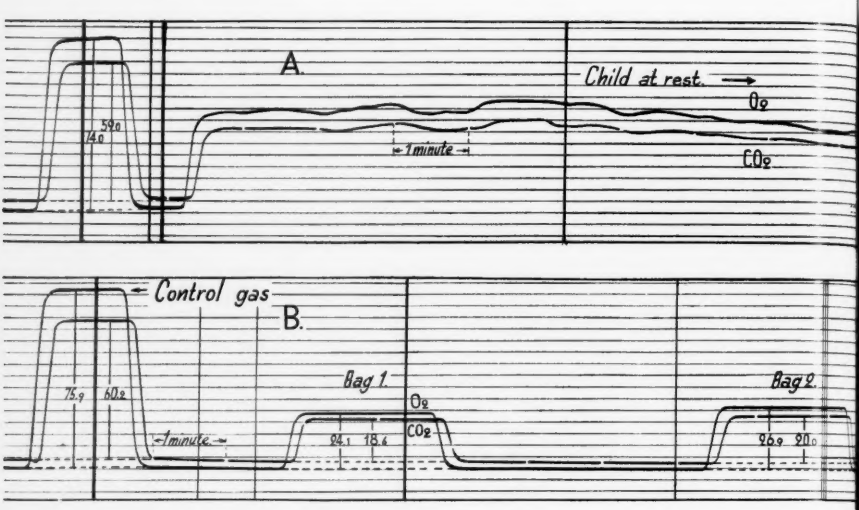


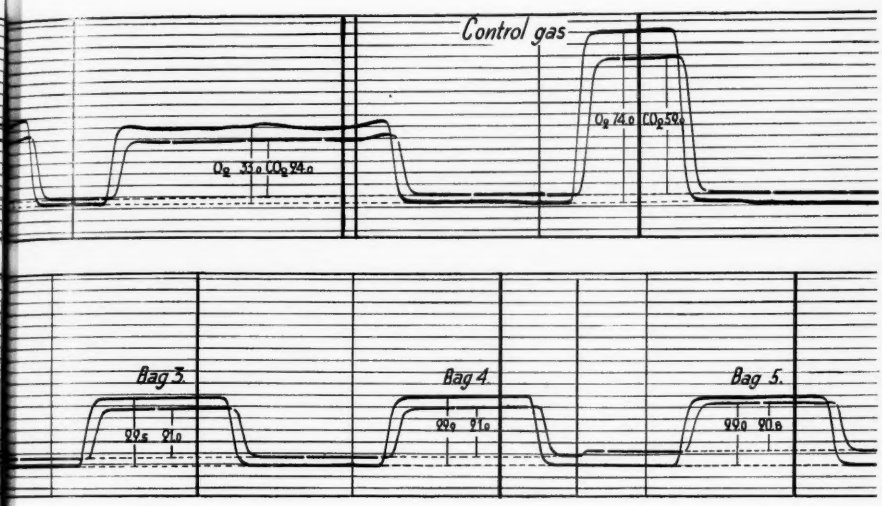
Fig. 41. *Typical record curves:*
(A) from a 6 months old infant, using the usual method for making the determination, (B) from a newborn, as a modification of the method.

Alcohol tests.

The method has been checked by alcohol tests. The arrangement for this is the same as that used in the closed method, except for a glass funnel which is placed over the alcohol flame, through

Table 11. *Results of alcohol test using the open circuit system.*

CO_2			O_2			RQ	
Theoretical ml. mts.	Found ml, mts.	Error percent.	Theoretical ml. mts.	Found ml. mts.	Error percent.	Found	Error percent.
403	394	— 2.2	605	585	— 3.3	0.670	+ 0.5
400	402	+ 0.5	600	598	— 0.3	0.674	+ 1.1
409	405	— 0.8	613	603	— 1.6	0.673	+ 0.9
420	414	— 1.4	630	620	— 1.6	0.668	+ 0.2
403	410	+ 1.6	605	600	— 0.8	0.684	+ 2.5
406	413	+ 1.5	612	614	+ 0.3	0.675	+ 1.2
400	403	+ 0.8	600	579	— 3.5	0.690	+ 4.4
405	405	± 0.0	606	589	— 2.8	0.688	+ 3.1
Mean of error		± 0			— 1.7		+ 1.7



which fresh air has been sucked. The noted results will be found in Table 11. To judge from the present findings, the reliability of the method is as good as American open circuit apparatuses used in recent years (see Table 1, page 28).

Table 12. *Comparison between closed and open methods.*

Subject	O_2 consumption Using closed method lits./hr.	O_2 consumption Using open method lits./hr.	Diff. between closed and open method lits./hr.	Diff. between closed and open method per cent
A. L.	2.86	2.89	+ 0.03	+ 1.04
L. S.	2.78	2.82	+ 0.04	+ 1.43
M. P.	2.68	2.73	+ 0.05	+ 1.85
G. P.	3.25	3.10	— 0.15	— 4.74
R. B.	2.27	2.21	— 0.06	— 2.68
U. N.	1.39	1.36	— 0.03	— 2.18
C. N.	1.69	1.68	— 0.01	— 0.6

A comparison with the present method according to the closed method.

A number of children have been investigated both with this open method and with the closed method, as described earlier. The investigation conditions have been identical. The results (see Table 12) show a good uniformity.

Investigations on newborns.

A number of newborns have been examined by this open method at the Obstetric Clinic of the Caroline Hospital. In this connection the modification as described above has been used. The children have been investigated immediately after delivery (within 10 minutes), 2 hours later, then again 2 hours later and afterwards as a rule once daily during the first week of life. All children have been fed from the mother's breast, and are put to the breast for the first time 12 hours after birth, and then 6 times per 24 hours. The purpose of this is to find a method suitable for this type of patient.

A graphic account of the oxygen consumed, carbon dioxide produced and calculated respiration quotient for each individual will be seen in Fig. 42. All cases disclose similar courses for the respiratory exchange, i.e. a high value immediately after birth and after that a decrease during the first 4 hours, then usually an increase on the following day. During the following days the level becomes more even. The respiration quotient decreases at first and, then rises up to about 0.86, i.e. the mean value for the time following the newborn period, according to earlier investigations (see the compilation in Fig. 23, page 75). This increase in the respiratory quotient synchronizes with the time when ordinary amounts of breast-milk are being given to the infant.

The investigations have been easily performed. They have not troubled the children.

The investigation material is too small to justify any far-

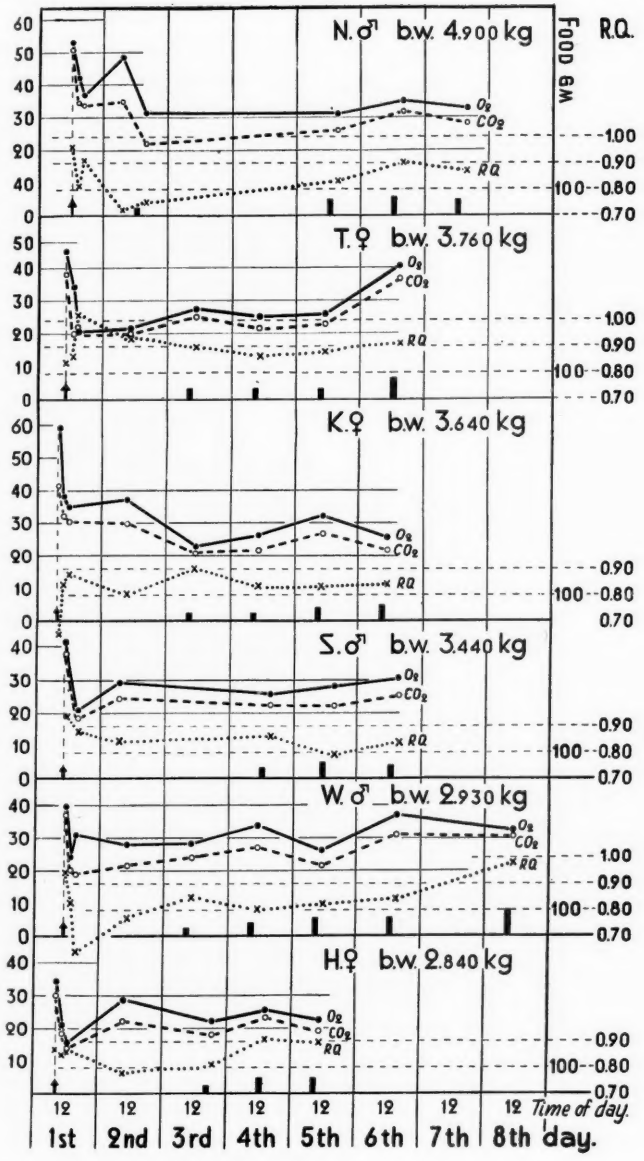


Fig. 42. *Diagrams showing the results from determinations made on newborns during the first week of life.*

reaching conclusions being made, regarding the energy metabolism during the newborn period. Also, the condition of each child must in this connection be carefully observed, owing to the rapid changes in the condition characteristic of the newborn period.

SUMMARY

The methods for determining the energy metabolism under standard conditions has long been established in clinical work, as far as adults and older children are concerned. However it has not proved applicable to infants.

A great many scientists have made a study of the energy metabolism in infants, since the initiation of metabolic research, and this has been mainly by means of indirect calorimetry. A brief survey of the literature is submitted.

A careful examination of the methods applied, has shown that none are immediately applicable to routine clinical practice.

A new method has been devised by the author. It is hoped, that its simplicity should render it clinically useful. It is designed on the principle of the closed system. To the *Krogh's* spirometer, which is the apparatus used most commonly in Scandinavia for making determinations on adults and older children, is coupled a simple respiration chamber and a circulation pump. The carbon dioxide produced by a subject is reabsorbed by a soda lime filter built into the spirometer, Thus, only the oxygen consumption of a child will be determined. This is obtained from the gradual diminution of the volume of the system which is continuously registered on the kymograph of the spirometer.

The decrease in the volume in the system will equal the oxygen consumption of a subject, only when gas equilibrium (temperature, humidity and carbon dioxide concentration) has been achieved. For this reason, a preliminary period of about half an hour is required. Because of the fact that an infant consumes considerably less oxygen per time unit than adults or older children the actual observation period must be prolonged for about 30 minutes, in

order to secure sufficient accuracy. Accordingly, an investigation will take about an hour. It can be easily performed by a single person.

On account of the construction of the apparatus, no special contrivance for registering the movements of a subject is necessary. Such movements will be obtained from the kymographic curves.

A constant fall in the kymographic curve, while a subject is at rest, will serve as a criterion of gas equilibrium in the system, and the decrease in volume will equal the oxygen consumption of a subject. This has been checked in 80 investigations, against the temperature in different parts of the system and the humidity in the respiration chamber.

The reliability of the apparatus has been checked by alcohol tests.

Suitable investigation conditions, including the physical condition of the subject, food and time of day have been discussed. It has been found advisable to make investigations on children in the morning or early forenoon, in a fasting condition, and in order to smothe the subjects during the period of the observations a dose of barbituric acid preparation is administered as a sedative. The preparation, hexobarbital, has been used in doses that have not affected the energy metabolism. The author's investigations have shown that there is a 24 hour rhythm in the energy metabolism. Consequently, it is essential that the investigations are performed at a certain time in the day, in order to establish comparable investigation conditions. The morning or early forenoon have been chosen in the present work.

The conditions applied to investigations on infants are not altogether comparable to those affecting adults and older children, i.e. the so-called basal conditions. Nor can they, in fact, be regarded as true basal conditions. Therefore, the values obtained by the present method have been stated to refer to standard and not basal metabolism.

The random error of the method, reflecting errors in the oxygen consumption and minor dissimilarities in the investigation conditions, as well as individual variations from day to day, has

been calculated from the differences between duplicate determinations. It has been found to remain at ± 0.073 lits. O_2/hr. At an oxygen consumption of 2 lits./hr., this will amount to ± 3.6 per cent and at 4 lits./hr. ± 1.8 per cent.

Investigations of the influence of food on the oxygen consumption of infants have shown that by the present method the so-called "specific dynamic effect" can be ascertained.

For an analysis of the energy metabolism from the oxygen consumption of the infants, a mean value of 15 per cent for protein metabolism has been applied, and a mean value of 0.86 for the respiratory quotient. The latter value has been obtained from a summation of values from investigations on infants available in the literature. The error introduced by this approximation yields a 95 per cent confidence interval to a maximal value of ± 2 per cent.

The range of applicability of the method is as follows: Determination of standard energy metabolism in infants aged from 1 week to 1 year, and if the child is small enough, up to 2 years. In order to secure satisfactory reliability in routine clinical work, children not exceeding a body weight of 2.5—3.0 kg. have been excluded. In addition, the respiratory quotient is taken to lie within normal variations.

By the present method, normal material consisting of 60 children aged from 8 days to 1 years has been investigated. Their physical development has been checked by means of a Swedish growth chart, worked out by the present author and co-workers. The material has disclosed no difference between boys and girls.

In order to correlate the energy metabolism with the body size, the latter must be represented by a suitable correlation factor. The correlation factors used in the literature have been examined.

The author has tested the correlation between the energy metabolism and the body weight in the general equation

$$C = W^b \cdot k$$

(C = Calories, W = weight, b and k = constants)

The exponent of the weight (b) has been found to be about 1 in the author's material, as well as in the available comparable

materials in the literature. This explains why the simple rule of expressing energy metabolism per kg. body weight has proved so useful in its application to infancy. In the older infants, the exponent *b* decreases and this change of the value of *b* becomes more obvius as the child approaches adolescence, to judge from *Benedict-Talbot's* big child material from birth to puberty. The exponent approaches more and more the ¾ found by *Kleiber*. This value applies to different-sized adult homeotherms, i.e. from mice to cattle. The higher exponent noted during infancy must be due partly to the influence of growth on the energy metabolism. This gradual change in the exponent *b* is observable already in infancy, and it invalidates to some extent the use of the body weight as a single correlation factor.

Nor is weight, as a single variable, suitable as a correlation factor, seeing that all individuals are assumed to possess e mean physical constitution. This will involve an approximation.

Since the relationship between weight and height gives an estimation of the physical constitution of an individual, it follows that when these two body measurements are combined they may constitute a better correlation factor with regard to the energy metabolism.

The relationship between the energy metabolism, on the one hand, and the weight and height, on the other, has been described by an equation of the form

$$C = W^b \cdot H^a \cdot k$$

(C = Calories, W = weight, H = height, b, a and k = constants.)

By attributing different values to the exponent *b* and *a*, a variety of different formulae have been obtained. This procedure has been tried in the present material, as well as in the only comprehensive material traceable in the literature, *Benedict-Talbot* and *Levine* et al.'s.

A multiple regression analysis has shown that this method is significantly better than using the weight or the height separately. The residual standard deviation has decreased to 5.1 per cent from 6.6 per cent, and from 9.5 per cent, when weight alone and height alone are applied as variables respectively. For the American material this is less pronounced, since the residual

standard deviation round the regression plane, expressed in the equation, is as big as 11.2 per cent.

This regression in fact, does not even appear to be quite rectilinear, as judged from a constructed 3-dimensional model with logarithmic axes for each material. For the older infants, the regression plane betrays a certain curvilinear tendency towards the weight-height plane. The regression equation obtained by the abovementioned procedure must therefore be handled with the same care in the case of the older infants.

An attempt has been made to extend the applicability of one single regression equation to the whole infant period.

In order to escape this approximation for the correlation factor, a new one has been tried, viz., the electrical capacitance of the body surface. With a view to practical use, a nomogram has been devised according to which the "capacitance surface" is obtained from weight and height. As far as the author's material is concerned, the relationship between the energy metabolism and the abovementioned "capacitance surface" is found to be rectilinear. The residual standard deviation is about the same size, as in the multiple regression analysis.

Both these procedures have been employed in the compilation of values from the author's normal material.

Among normal values for infants recorded in the literature, under conditions approximately similar to those in the author's material, *Kestner-Knipping's* normal tables stand alone and are based on an age factor and a weight factor, together with *Levine-Marples'* analysis of *Benedict-Talbot's material* from 1921.

In a comparison with the abovementioned materials, the author's material reveals a fair conformance to the American material, as far as small infants are concerned, while in the case of the older infants, the author's material gives about 12 per cent lower values. When all the infant period is taken together, the difference between the two materials is significant. Compared with the values recorded by *Kestner-Knipping,* the value for the youngest children in the author's material are higher and for the older infants they are approximately similar.

The difference from the American material could be explained

easily by the dissimilarities in the investigation procedures. The present author investigated the children in a fasting state in the morning or early forenoon, while the Americans have given the children a small meal before the investigations, and they performed their observations during the middle of the day or somewhat later. Both of the latter conditions will increase the energy metabolism. *Benedict-Talbot's* child material from birth to puberty, emphasizes in its entirety the significance of this difference in the investigation conditions. At a body weight of about 16 kg., the investigation conditions are more similar to those used for infants by the author. At this point, their values fall.

The difference between the younger infants and *Kestner-Knipping's* standard values is more difficult to judge, since no original values for the energy metabolism have been given and there is no data regarding their investigation conditions.

The values obtained from the author's normal material have been used to form two nomograms for predicting the standard energy metabolism for infants from 1 week old to 1 year old. One based on the multiple regression equation, the other on the "capacitance surface". In both instances, the body size of a child is represented by its weight and height.

As an application of the method, infants with untreated and treated hypothyroidism were examined.

It is not quite suitable to use the abovementioned method employing the closed system to determine the energy metabolism of newborns. Since the respiratory quotient varies during the first week of life, both the oxygen consumption and the carbon dioxide should be determined. The energy metabolism of these infants is low and the reliability of this method decreases the smaller the child is. Besides this the variations in the respiratory exchange occur so rapidly, that the observation period has to be as short as possible. To overcome the abovementioned difficulties the author has devised an open circuit method, in which a small respiration hood covers the child's head alone. The gas analysis is recorded continuously by a "Gaswechselschreiber" according to *Rein*. Alcohol tests have confirmed the reliability of this

method. Determinations made on children more than one week old have shown uniform results, when either the open or closed systems are employed.

Determinations made on newborns have shown that the method is suitable for studying the energy metabolism in newborns. Some examples are given.

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APPENDIX

App. 1. *Schematic representation of results obtained from normal infants.*

Description of subject.						Last feeding		Premedication amt. of tablet[3] (0.25gm)	Chamber small standard	Temp. in degrees cent. in spirom.	Time at obs. period	O_2 consump. lits./hr.	Diff. in O_2 consump. between two observations on the same subj.[4]			Energy metabolism calculated from O_2 consump.	
Number	Sex	Birth-weight kg.	Age mth.:wk.	Present weight kg.	Present height cm.	Type of food[1]	When ingested mts.[2]						Exper. condits. unchanged.	Interval diff. following feeding	Different time of day	Cals./hr.	Cals./24hrs.
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	F	3.16	0:1	2.83	46.5	Br.	120	—	sm.	18.5	9.30	1.38					
			0:1	2.84	46.5	Br.	120	—	sm.	18.5	9.30	1.30	2	+0.08			
2	M	4.80	0:2	4.33	54	Br.	165	1/4	sm.	18.0	9.45	1.98				9.55	229
3	M	4.43	0:2	4.09	53	Frm.	175	1/4	st.	18.0	10.05	2.28					
			0:3	3.80	53	Frm.	175	1/4	st.	18.0	9.55	2.44	3	−0.16			
			0:3	3.91	53	Frm.	185	1/4	st.	18.7	11.05	2.38	1	+0.06			
4	M	3.71	0:3	3.69	54	Br.	140	1/4	st.	20.5	9.30	1.75					
			0:3	3.70	54	Br.	125	1/4	st.	19.8	10.00	1.86	1	−0.11			
			0:3	3.80	54	Br.	220	1/4	sm.	19.2	10.40	1.63		1 +0.23		7.86	189
5	M	3.40	0:3	3.15	54	—	—	1/4	sm.	21.5	9.15	1.44				6.95	167
6	F	3.66	0:3	3.45	51.5	—	—	1/4	sm.	19.8	9.05	1.50				7.24	174
7	M	3.75	0:3	3.48	52	Br.+Frm.	75	—	sm.	20.0	15.45	1.51					
			1:0	3.57	52	Br.+Frm.	70	1/3	sm.	19.0	12.10	1.85			4 −0.34		
8	F	3.56	0:3	3.73	55	Frm.	120	1/4	sm.	19.0	16.00	1.92					
			0:3	3.73	55	Frm.	120	1/4	st.	19.5	16.10	2.07	1	−0.15			
9	M	3.40	1:0	3.78	52	—	—	1/4	st.	21.2	9.50	1.77					
			1:1	3.84	52	—	—	1/4	sm.	22.0	9.10	1.73	3	+0.04			
			3:0	5.06	58	—	—	3/8	st.	21.0	9.15	2.43				11.72	281
10	M	3.40	1:0	3.41	49.5	—	—	1/4	st.	20.4	9.25	1.44				6.95	167
11	F	3.48	1:0	3.74	52	—	—	1/2	st.	19.4	9.20	1.78				8.59	206
12	M	3.68	1:0	3.75	52	—	—	1/4	st.	21.0	10.25	2.02					
			1:0	3.84	52	—	—	1/4	st.	19.7	10.30	1.88	5	+0.14		9.07	218
13	M	3.20	1:2	3.71	—	Br.+Frm.	70	—	sm.	23.7	11.30	2.10					
			1:3	3.72	—	Br.+Frm.	75	—	sm.	21.8	12.15	1.96	1	+0.14			
14	M	3.98	1:2	4.40	58	Br.+Frm.	195	1/4	sm.	19.8	14.15	2.47					
			1:2	4.50	58	Br.+Frm.	245	1 3	sm.	19.7	11.05	2.61			1 −0.14		
15	F	3.88	1:3	3.61			90	1/2		19.0	12.45						

13	M	3.20	1:2	3.71	—	Br.+Frm.	75		sm.	21.8	12.15	1.96	1	+0.14				
			1:3	3.72		Br.+Frm.	75		sm.									
14	M	3.08	1:2	4.40	58	Br.+Frm.	195	1/4	sm.	19.8	14.15	2.47						
			1:2	4.50	58	Br.+Frm.	245	1 3	sm.	19.7	11.05	2.61			1	−0.14		
15	F	2.88	1:3	3.64		Br.+Frm.	80	1/3	sm.	19.0	12.10	1.93						
			2:0	3.73	53	Br.+Frm.	99	3 4	sm.	19.5	16.00	1.84			8	+0.09		
16	F	3.55	1:3	4.14	56	Br.	75	1/2	sm.	20.2	17.30	1.71						
			1:3	4.21	56	Br.	230	1/2	sm.	18.4	10.50	1.80					8.69	208
			1:3	4.32	56	Frm.	105	1 2	sm.	21.4	16.45	1.77	8	−0.06				
17	M	3.38	1:3	4.45	58	Br.+Frm.	150	3/4	sm.	18.1	9.30	1.91						
			1:3	4.44	58	Br.+Frm.	156	3/4	sm.	18.7	9.45	1.84	1	+0.07				
			1:3	4.37	58	Br.+Frm.	250	3 4	sm.	18.9	15.10	1.72						
			1:3	4.37	58	Br.+Frm.	255	3/4	sm.	18.8	11.15	1.98			1	−0.26		
			2:3	5.05	59	Br.+Frm.	120	1	sm.	19.8	17.00	2.21						
			2:3	5.14	59	Br.+Frm.	150	1	sm.	19.9	17.30	2.15	6	+0.06				
			3.0	5.20	59	Br.+Frm.	120	1	sm.	18.3	13.00	2.48			1	−0.33		
18	M		1:3	3.72	56.5	Frm.	85	1 4	sm.	21.0	16.25	1.56						
			1:3	3.76	56.5	Frm.	55	1/4	sm.	20.8	12.35	1.69			2	−0.13		
19	M		2:0	4.65	58	Fas.	—	1 2+1/4	sm.	19.2	9.15	1.90					9.17	220
20	F	4.18	2:0	5.54	62	Fas.	—	1/4	sm.	19.5	9.45	2.27					10.95	263
21	M	3.89	2:1	4.57	57	Fas.	—	1/4	st.	20.0	8.40	2.12					10.23	245
22	M	3.35	2:1	4.51	57	Fas.	—	1/4	st.	20.0	9.15	1.86					8.97	215
23	M	3.47	2:1	4.46	57.5	Fas.	—	1/2	st.	16.4	10.45	2.03					9.79	235
24	F	4.28	2:2	5.24	58	Fas.	—	1/4	st.	20.0	9.45	2.26					10.90	262
25	F	3.73	2:2	4.61	60	Fas.	—	1/4	st.	19.5	9.45	1.96					9.46	227
			4:0	5.67	64	Frm.	105	1 2	st.	19.5	16.15	2.62						
			4:0	5.67	64	Frm.	315	1/2	st.	20.6	16.35	2.49	1	+0.13				
26	M	3.27	2:2	3.84	52.5	Fas.	—	3/4	sm.	19.0	8.30	1.65					7.96	191
27	M	3.65	2:2	5.30	60.5	Fas.	—	3/8	st.	22.0	9.20	2.52					12.16	292

1) Br. = breast milk. Frm. = cow's milk formula. Fas. = fasting during 5-10 hrs.
2) Time of ingestion = mts. elapsed between feeding and middle of obs.period.
3) Tab. Hexobarbital.
4) The two observations are connected with a vertical line. The interval in days appears on the left and on the right the diff. in O_2 consump. in lits./hr. (Obs.1 − Obs.2).

App. I. *Schematic representation of results obtained from normal infants. (continued)*

Description of subject.						Last feeding		Premedication amt. of tablet[3] (0.25gm)	Chamber small standard	Temp. in degrees cent. in spirom.	Time at obs. period	O_2 consump. lits./hr.	Diff. in O_2 consump. between two observations on the same subj.[4]			Energy metabolism calculated from O_2 consump.	
Number	Sex	Birthweight kg.	Age mth.:wk.	Present weight kg.	Present height cm.	Type of food[1]	When ingested mts.[2]						Exper. condits. unchanged.	Interval diff. following feeding	Different time of day	Cals./hr.	Cals./24hrs.
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
28	M	3.60	2:2	4.01	57	Br.+Frm.	125	1	sm.	19.0	17.35	2.02					
			2:2	4.02	58.5	Br.+Frm.	170	1	sm.	20.0	17.50	1.99	1 +0.03				
			2:2	4.07	58.5	Br.+Frm.	130	1	sm.	19.8	12.20	2.22			2 −0.20		
			2:2	3.60	58.5	Frm.	180	1	sm.	19.5	18.00	2.03	3 −0.04				
			2:2	3.60	58.5	Frm.	90	1	sm.	18.6	12.30	2.21					
			2:2	3.60	59	Frm.	90	1 1/2	sm.	18.0	12.30	2.08	2 +0.13				
			3:0	4.25	59	Frm.	120	1 3/4	sm.	18.0	17.00	2.30					
			3:0	4.40	59	Frm.	75	1 3/4	sm.	19.3	15.45	2.41					
			3:0	4.40	59	Frm.	225	1 3/4	sm.	18.5	15.45	2.19		1 +0.22			
			3:0	4:40	59	Frm.	135	1 3/4	sm.	19.0	12.25	2.44			5 −0.14		
			3:0	4.44	59.5	Fas.	—	1 3/4	sm.	18.0	8.40	2.17					
29	M		2:2	5.17	60	Fas.	—	1/2	st.	22.0	8.20	2.27				10.95	263
30	F	2.70	2:3	4.50	59.5	Frm.	60	1/2	sm.	20.0	16.15	2.30					
			2:3	4.52	59.5	Frm.	200	1/2	sm.	20.1	14.20	2.05					
			2:3	4.54	59.5	Frm.	195	1/2	sm.	21.2	14.15	1.99	1 −0.06				
			2:3	4.67	59.5	Fas.	—	1/2	sm.	19.5	10.45	2.18				10.52	252
31	M	3.46	2:3	4.23	53	Fas.	—	1/4	st.	20.7	9.35	1.81				8.73	210
32	F	2.67	3:0	4.35	56.5	Fas.	—	1/3	st.	20.2	9.30	1.99				9.60	230
33	M	4.58	3:1	6.49	65	Fas.	—	3/4	st.	22.6	9.05	2.78				13.41	322
34	M	5.70	3:2	5.70	64	Fas.	—	1/2	st.	24.0	9.15	2.77				13.36	321
35	M	2.83	3:2	5.08	60.5	Fas.	—	1/2	st.	21.2	9.25	2.45				11.82	284
36	F	3.61	4:0	5.40	62	Fas.	—	1/4	st.	22.0	8.55	2.39				11.52	277
37	M	3.24	4:0	6.75	67	Frm.	260	1 2+1/4	sm.	20.0	15.05	2.82					
			4:0	6.78	67	Frm.	100	3/4	sm.	21.7	15.40	3.06		4 −0.24			

37	M	3.24	4:0	6.75	67	Frm.	260	1 2 + 1 4	sm.	20.0	15.05	2.82							
			4:0	6.78	67	Frm.	100	3 4	sm.	21.7	15.40	3.06		4 —0.24					
			4:0	6.81	67	Frm.	210	3 4	sm.	21.0	14.30	2.71	5 + 0.11						
			4:0	6.82	67	Frm.	110	3 4	sm.	19.8	12.35	3.09			2 —0.03				
			4:0	6.82	67	Frm.	280	3 4	sm.	19.8	15.40	2.94		3 + 0.12					
			4:0	6.93	67	Frm.	300	3 4	sm.	19.0	12.00	3.00			5 —0.6				
38	M	3.54	4:0	5.59	64.5	Fas.	—	1 2	st.	22.4	8.55	2.43					11.72	281	
39	M	3.15	4:1	6.69	65.5	Fas.	—	1 2	st.	18.6	9.45	3.08					14.86	357	
			4:1	6.69	65.5	Fas.	—	1 2	st.	18.0	9.30	2.88	1 + 0.20						
40	F	3.78	4:1	6.32	63.5	Fas.	—	1 2	st.	21.3	10.20	2.92							
			4:1	6.04	63.5	Fas.	—	1 2	st.	21.2	9.20	3.00	2 —0.08				14.48	347	
41	F	2.95	4:1	5.02	59.5	Fas.	—	1 4	st.	19.4	9.15	2.49					12.01	288	
42	M	4.30	4:2	7.15	67	Fas.	—	1 2	st.	20.2	8.50	2.86					13.80	331	
			4:3	7.28	67	Fas.	—	1 2	st.	19.2	9.15	2.94	10 —0.08						
43	M	3.74	4:2	6.25	63.5	Fas.	—	1 2	st.	24.0	9.15	2.88					13.90	334	
44	F	3.40	4:3	7.16	62.5	Fas.	—	3 4	st.	22.5	9.10	2.78					13.41	322	
45	M	3.20	5:0	7.29	70	Frm.	200	1 2	st.	21.1	10.20	2.86					13.80	331	
46	F	3.52	5:0	6.20	59	Frm.	110	1 2	st.	20.2	16.20	2.04							
47	F	2.75	5:1	5.99	62.5	Frm.	200	1 2	st.	18.5	10.20	2.78					13.41	322	
48	M	3.22	5:1	7.23	66	Fas.	—	1 2	st.	21.0	9.45	3.24					15.63	375	
			5:1	7.27	66	Fas.	—	1 2	st.	21.0	9.10	3.04	3 + 0.20						
49	M	4.08	5:1	6.60	65	Fas.	—	3 8	st.	20.2	8.35	3.05					14.72	353	
50	F	2.87	5:1	5.86	61	Fas.	—	1 2	st.	23.0	9.10	2.90					13.99	336	
51	F	4.31	5:2	7.38	67	Fas.	—	1 2	st.	19.8	9.40	3.12					15.05	361	
			5:2	7.38	67	Fas.	—	1 2	st.	19.8	10.07	3.16	1 —0.04						
52	F	3.97	5:2	7.32	66.5	Fas.	—	3 4	st.	22.4	9.35	3.08					14.86	357	
53	M	3.45	5:3	7.10	66	Fas.	—	3 4	st.	19.0	9.53	3.00					14.48	347	

1) Br. = breast milk. Frm. = cow's milk formula. Fas. = fasting during 5-10 hrs.
2) Time of ingestion = mts. elapsed between feeding and middle of obs.period.
3) Tab. Hexobarbital.
4) The two observations are connected with a vertical line. The interval in days appears on the left and on the right the diff. in O_2 consump. in lits. / hr. (Obs 1 — Obs. 2).

App. I. *Schematic representation of results obtained from normal infants.* *(continued)*

Description of subject.						Last feeding		Premedication amt. of tablet[3] (0.25gm)	Chamber small standard	Temp. in degrees cent. in spirom.	Time at obs. period	O_2 consump. lits./hr.	Diff. in O_2 consump. between two observations on the same subj.[4]			Energy metabolism calculated from O_2 consump.	
Number	Sex	Birthweight kg.	Age mth.:wk.	Present weight kg.	Present height cm.	Type of food[1]	When ingested mts.[2]						Exper. condits. unchanged.	Interval diff. following feeding	Different time of day	Cals./hr.	Cals./24hrs.
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
54	F	4.36	6.0	8.23	73	Fas.	—	1/4	st.	22.0	9.40	3.70				17.85	428
			6:0	8.26	73	Fas.	—	1/4	st.	21.3	9.15	3.74	1 —0.04				
55	M	3.40	6:0	7.27	69	Fas.	—	3/4	st.	21.4	9.05	3.36				16.21	389
56	F	2.73	6:0	6.57	63	Fas.	—	3/4	st.	21.2	9.10	2.76				13.32	320
57	F	3.09	6:1	6:56	64.5	Fas.	—	1/2	st.	21.0	9.35	2.59				12.50	300
58	M	3.64	6:2	7.09	67	Fas.	—	1/4	st.	21.0	9.50	2.88				13.90	334
59	F	3.53	6:3	6.92	65.5	Fas.	—	3/4	st.	21.6	8.55	2.97				14.33	344
60	F	3.41	6:3	7.41	65.5	Fas.	—	3/4	st.	22.6	9.25	2.86				13.80	331
61	F	3.99	6:3	7.35	65	Fas.	—	3/4	st.	23.8	8.30	3.26				15.73	378
62	M	3.72	7:1	9.40	72	Fas.	—	1	st.	22.8	9.20	3.96				19.11	459
63	M	3.99	7:1	9.45	74	Fas.	—	1	st.	22.0	9.35	3.76				18.14	435
64	F	3.79	7:2	8.30	70.5	Fas.	—	3/4	st.	21.8	9.05	3.29				15.87	381
65	M	4.07	7:3	8.76	71	Fas.	—	1	st.	21.8	9.15	3.85				18.58	446
66	M	3.37	8:1	8.28	73	Fas.	—	3/4	st.	21.9	8.55	3.46				16.69	401
67	M	3.22	9:0	8.86	76	Fas.	—	1	st.	21.4	9.15	3.62					
68	F	3.40	9:1	9.62	73.5	Fas.	—	1	st.	21.9	8.40	4.18				20.17	484
69	M	3.45	9:2	9.59	74	Fas.	—	1/2	st.	20.0	9.45	3.94				19.01	456
70	F	2.31	9:3	9.14	74	Fas.	—	1	st.	22.0	9.15	3.68				17.76	426
71	M	3.75	10:1	10.13	74	Fas.	—	1	st.	22.0	9.20	4.37				21.09	506
72	M	3.10	10:4	8.91	69.5	Fas.	—	1	st.	22.0	9.40	4.10				19.78	475

1) Br. = breast milk. Frm. = cow's milk formula. Fas. = fasting during 5-10 hrs.
2) Time of ingestion = mts. elapsed between feeding and middle of obs.period.
3) Tab. Hexobarbital.
4) The two observations are connected with a vertical line. The interval in days appears on the left and on the

3) Tab. Hexobarbital.

4) The two observations are connected with a vertical line. The interval in days appears on the left and on the

App. II. *Schematic representation of results obtained from prematures.*

Description of subject.						Last feeding		Premedication amt. of tablet[3] (0.25gm)	Chamber small standard	Temp. in degrees cent. in spirom.	Time at obs. period	O_2 consump. lits./hr.	Diff. in O_2 consump. between two observations on the same subj.[4]			Energy metabolism calculated from O_2 consump.	
Number	Sex	Birth-weight kg.	Age mth.:wk.	Present weight kg.	Present height cm.	Type of food[1]	When ingested mts.[2]						Exper. condits. unchanged.	Interval diff. following feeding	Different time of day	Cals./hr.	Cals./24hrs.
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Pr1	M	1.90	1:0	2.51	42	Br.	275	1/4	sm.	19.2	11.15	1.89					
			1:1	2.52	49	Br.	120	1/2	sm.	19.9	12.30	1.96		5 —0.07			
Pr2	F	1.89	2:1	2.85	47	Frm.	240	1/4	sm.	16.5	15.00	1.50					
			2:1	2.85	47	Frm.	95	—	sm.	18.0	17.05	1.68					
			2:1	2.88	47	Frm.	135	1/4	sm.	20.4	16.30	1.44					
			2:2	3.05	47	Frm.	260	1/4	sm.	20.0	15.20	1.43	8 +0.07	7 +0.25			
			2:2	3.20	47	Frm.	210	1/4	sm.	19.4	14.30	1.57	4 —0.14	6 +0.01			
Pr3	M	1.02	2:3	2.75	46	Br.	110	1/4	sm.	20.0	9.05	1.41					
			2:3	2.75	46	Br.	145	—	sm.	20.1	9.20	1.59	1 —0.18				
Pr4	M	1.35	3:2	3.70	52.5	Fas.	—	1/4	st.	18.6	9.00	1.51				7.29	175
			3:2	3.82	52.5	Fas.	—	1/4	sm.	19.4	10.05	1.55	6 —0.04				
Pr5	M	2.00	3:2	3.94	56	Fas.	—	1/4	st.	18.8	8.55	2.16				10.42	250
Pr6	M	2.23	7:1	7.46	66.5	Fas.	—	3/4	st.	22.6	8.55	3.34				16.12	387
Pr7	M	1.12	10:2	6.48	67	Fas.	—	3/4	st.	21.8	8.40	3.06				14.77	354
Pr8	M	1.92	10.2	9.52	75	Fas.	—	1	st.	22.0	9.15	3.88					

1) Br. = breast milk. Frm. = cow's milk formula. Fas. = fasting during 5-10 hrs.

2) Time of ingestion = mts. elapsed between feeding and middle of obs.period.

3) Tab. Hexobarbital.

4) The two observations are connected with a vertical line. The interval in days appears on the left and on the right the diff. in O_2 consump. in lits./hr. (Obs. 1 — Obs. 2).

App. III. *Schematic representation of results obtained from miscellaucous series of cases.*

Description of subject.						Last feeding		Premedication amt. of tablet[3] (0.25gm)	Chamber small standard	Temp. in degrees cent. in spirom.	Time at obs. period	O_2 consump. lits./hr.	Diff. in O_2 consump. between two observations on the same subj.[4]			Energy metabolism calculated from O_2 consump.	
Number	Sex	Birth-weight kg.	Age mth.:wk.	Present weight kg.	Present height cm.	Type of food[1]	When ingested mts.[2]						Exper. condits. unchanged.	Interval diff. following feeding	Different time of day	Cals./hr.	Cals./24hrs.
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
P1	M	5.00	0:1	4.34	74	Fas.	—	—	st.	19.0	8.50	1.55					
			0:1	4.36	74	Fas.	—	—	st.	18.0	8.50	1.51	1 + 0.04				
P2	M	3.08	0:1	2.88	49	Fas.	—	1/4	st.	22.4	10.30	1.21					
			0:2	2.70	49	Fas.	—	1/4	st.	22.3	10.15	1.24	3 — 0.03				
P3	F	3.43	0:3	2.87	49	Frm.	260	1/3	sm.	19.5	11.20	1.43					
			0:3	2.85	49	Frm.	75	1/2	sm.	20.0	12.20	1.82		3 — 0.39			
P4	F	3.62	1:3	3.95	55	Frm.	105	1/2	st.	20.4	16.45	2.06					
			1:3	3.99	55	Frm.	90	1/2	sm.	19.4	16.30	1.87	3 + 0.19				
P5	F	3.51	2:0	4.21	—	Frm.	225	1/4	st.	27.0	14.45	2.10					
			2:1	4.39	—	Frm.	215	1/4	st.	22.0	14.40	2.19	9 — 0.09				
P6	F	3.30	2:3	3.56	54	Fas.	—	1/4	st.	21.8	9.20	1.38					
			2:3	3.60	54	Fas.	—	1/4	st.	20.6	9.25	1.35	1 — 0.03				
P7	M	3.88	3:0	4.81	72	Frm.	165	3/4	sm.	18.5	9.45	2.12					
			3:0	4.76	72	Frm.	135	3/4	sm.	18.9	10.15	2.21	2 — 0.09				
P8	M	3.70	3:1	5.21	60	Frm.	220	1/4	st.	27.0	14.40	2.45					
			3:1	5.19	60	Frm.	240	1/4	st.	26.3	14.55	2.41	1 + 0.04				
			4:3	6.10	64	Frm.	165	1/4	st.	21.1	9.40	2.97					
			4:3	6.10	64	Frm.	175	1/4	st.	21.0	10.05	3.10	3 — 0.13				
P9	M	1.53	3:1	3.34	52	Fas.	—	1/4	sm.	18.0	10.40	1.64					
			3:1	3.36	52	Fas.	—	1/4	st.	19.0	10.20	1.64	2 + 0				
P10	M	4.12	3:3	5.16	64	Frm.	110	1 3 4	sm.	20.3	17.00	2.35					
			3:3	5.08	64	Frm.	150	1 3 4	sm.	19.8	16.30	2.33	1 + 0.02				
P11	F	2.59	6:2	6.88	65.5	Fas.		1 4	st.	20.5	8.25	2.91					
			6:2	6.88	65.5	Fas.		1 4	st.	21.0	8.05	3.14	1 — 0.23				
P12	M	3.00	7:0	7.47	66	Fas.		1 2	st.	19.0	9.10	2.62					
			7:0	7.18	66	Fas.		1 2	st.	17.8	9.30	2.72	1 — 0.10				

			6:2	6.88	65.5	Fas.		1/4	st.	21.0	8.05	3.14	1	0.23
P12	M	3.60	7:0	7.47	60	Fas.		1/2	st.	19.0	9.10	2.62		
			7:0	7.18	60	Fas.		1/2	st.	17.8	9.30	2.72	1	−0.10
P13	M	4.50	7:1	12.00	74	Fas.	—	1/2	st.	21.4	9.40	4.57		
			7:1	12.00	74	Fas.	—	1/2	st.	21.0	10.05	4.58	1	−0.01
P14	F	3.55	8:0	7.06	70	Frm.	—	1/2	st.	20.0	10.05	3.65		
			8:0	6.91	70	Frm.	—	1/2	st.	20.2	10.20	3.64	1	+0.01
P15	M	3.56	10:0	8.41	69.5	Fas.	—	3/4	st.	17.0	9.30	3.49		
			10:0	8.40	69.5	Fas.	—	3/4	st.	19.0	9.30	3.51	3	−0.02
P16	F	3.70	10:3	8.11	74	Fas.	—	1/3	st.	14.0	8.50	4.10		
			10:3	8.11	74	Fas.	—	1/3	st.	18.6	8.50	3.96	1	+0.14
P17	F	2.62	11:0	8.29	72	Fas.	—	3/4	st.	19.1	10.00	3.48		
			11:0	8.20	72	Fas.	—	3/4	st.	18.6	10.00	3.46	5	+0.02
P18	F	1.78	1:0[5]	6.07	61	Fas.	—	1/4	st.	17.6	9.10	2.17		
			1:0	6.02	61	Fas.	—	1/4	st.	18.4	8.50	2.12	1	+0.05
P19	M	4.20	1:2	10.03	76	Fas.	—	1/2	st.	20.0	9.00	4.02		
			1:2	10.23	76	Fas.	—	1/2	st.	20.6	8.55	4.13	3	−0.11
P20	M	3.20	1:2	6.90	70	Fas.	—	3/4	st.	21.0	10.00	3.33		
			1:2	6.90	70	Fas.	—	3/4	st.	20.0	9.25	3.43	1	−0.10
P21	M	3.28	1:2	13.25	81	Fas.	—	3/4	st.	21.8	9.25	4.91		
			1:2	13.29	81	Fas.	—	3/4	st.	22.8	8.55	5.00	1	−0.09
P22	M	3.09	1:5	10.00	74	Fas.	—	1/2	st.	19.8	9.05	3.60		
			1:5	10.00	74	Fas.	—	1/2	st.	19.4	9.00	3.55	1	+0.05
P23	M	4.08	1:7	7.88	69.5	Fas.	—	1/2	st.	20.0	8.35	3.33		
			1:7	7.80	69.5	Fas.	—	1/2	st.	20.0	8.05	3.28	1	+0.05
P24	M	3.42	1:10	7.61	65	Fas.	—	1/2	st.	21.0	11.40	4.40		
			1:10	7.61	65	Fas.	—	1/2	st.	18.5	10.40	4.47	1	−0.07

1) Br. = breast milk. Frm. = cow's milk formula. Fas. = fasting during 5-10 hrs.
2) Time of ingestion = mts. elapsed between feeding and middle of obs.period.
3) Tab. Hexobarbital.
4) The two observations are connected with a vertical line. The interval in days appears on the left and on the right the diff. in O_2 consump. in lits. / hr. (Obs. 1 − Obs. 2).
5) Year: mth.

App. IV. *Schematic representation of results obtained from untreated and treated hypothyroids.*

Description of subject.						Last feeding		Premedication amt. of tablet[3] (0.25gm)	Chamber small standard	Temp. in degrees cent. in spirom.	Time at obs. period	O_2 consump. lits./hr.	Diff. in O_2 consump. between two observations on the same subj.[4]			Energy metabolism calculated from O_2 consump.	
Number	Sex	Birth-weight kg.	Age mth.:wk.	Present weight kg.	Present height cm.	Type of food[1]	When ingested mts.[2]						Exper. condits. unchanged.	Interval diff. following feeding	Different time of day	Cals./hr.	Cals./24hrs.
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	F	3.64	4:1	4.82	57.0	Fas.	—	3/8c	st.	18.4	9.40	1.35				6.50	Untreat.
			4:3	4.82	58.5	Fas.	—	3/8c	st.	18.8	8.30	2.34				11.30	Treat.
2	F	2.50	5:2	6.11	58.0	Fas.	—	1/2c	st.	21.0	9.00	1.87				9.02	Untreat.
3	F	3.45	9:2	8.61	70.0	Fas.	—	1/2c	st.	18.4	8.45	2.58				12.45	Untreat.
			1:10[5]	9.19	82.5	Fas.	—	1c	st.	18.6	9.00	4.10				19.80	Treat.
4	M	4.80	10:1	9.02	69.0	Fas.	—	1/2c	st.	20.0	9.15	2.18				10.50	Untreat.
			10:3	8.79	69.0	Fas.	—	1/2c	st.	21.7	9.15	3.63				17.50	Treat.
5	F	4.32	11:3	10.50	72.0	Fas.	—	1c	sm.	19.0	12.00	2.82				13.50	Untreat.
			1:0	10.61	72.0	Fas.	—	1c	sm.	17.0	10.35	2.77				13.30	Untreat.
			1:0:2	11.11	72.0	Fas.	—	3/4c	sm.	18.5	8.35	2.94				14.10	Untreat.
			1:1:2	10.14	72.0	Fas.	—	1c	sm.	19.7	10.30	3.97				19.26	Treat.
6	F	3.14	1:4:2	8.00	62.0	Fas.	—	1/4c	st.	20.1	10.40	2.31				11.15	Untreat.
			1:4:2	7.95	62.0	Fas.	—	—	st.	21.1	10.55	2.07				9.99	Untreat.
			1:6	8.10	68.0	Fas.	—	1c	st.	18.6	9.50	3.51				16.85	Treat.

1) Br. = breast milk. Frm. = cow's milk formula. Fas. = fasting during 5-10 hrs.
2) Time of ingestion = mts. elapsed between feeding and middle of obs.period.
3) Tab. Hexobarbital.
4) The two observations are connected with a vertical line. The interval in days appears on the left and on the right the diff. in O_2 consump. in lits./hr. (Obs 1 — Obs. 2).
5) Year: mth.

App. V. *A schematic summary of the litterature.*

Year of publication	Author: Using open method	Author: Using closed method	Description of apparatus	Determination of: O_2	Determination of: CO_2	Determination of: Urinary N_2	Investigations conditions: Time: Long	Time: Short: a. m.[1]	Time: Short: p. m.	Premedication	Food: Fasing	Food: Tea	Food: Small meal	Calculation from: O_2	Calculation from: CO_2	Calculation from: RQ[2]	Calculation from: Urinary N_2	Number of cases: Premature: Newborn ↓ <1 wk	Number of cases: Premature: >1 wk ↓ < year	Number of cases: Full term: Newborn ↓ <1 wk	Number of cases: Full term: >1 wk ↓ < 1 year	Number of cases: Underweigt	Comments
1843		(Scharling)			×																	
1949		Regnault-Reiset	×	×	×																	
1862		Pettenkoffer-Voit	×		×																	
1870		(Liebermeister)	×		×																	
1877	Forster		×		×			×							×						2		
1894		Mensi	×	×	×			×						×	×	×			1	4			
1896		Scherer	×	×	×			×						×	×	×				85			Low RQ values
,,	(Wolpert)		×		×																		
1898	Rubner-Heubner		×		×		×						×		×						1		
1899	Rubner-Heubner				×		×						×		×						1	1	
1900		Poppi	×	×	×			×						×	×	×					7		High RQ values
1902		Babák	×	×	×			×						×	×	+				7			
1904	Hasselbalch		×	×	×			×			×			×	×	+		3		17			
1904	Rubner-Heubner				×		×						×		×	+					1		
1905																							
1908		Zuntz-Oppenheimer	×	×	×																		
,,		Schlossman-Murschhauser	×	×	×			n			×			×	×	×				1			The influence of food
,,		Weiss	×	×	×			×						×	×	+		3		9	6		
1909		Schlossmann-Murschhauser		×	×			n			×			×	×	×					1		
,,	v. Bergmann				×		×						×								1		Myxoedema
1910	Bahrdt-Edelstein		×		×		×						×										
,,	Birk-Edelstein				×								×		×					1			
,,		Schlossmann-Murschhauser		×			×	n				×		×	×	×					3		
1911		Schlossmann-Murschhauser		×	×			n			×			×		×						2	The influence of temp.
,,	Niemann		×		×		×						×		×						2		
,,		Carpenter-Murlin	×												×					×			
1912		(Williams) Benedict	×	×	×																		
,,		Howland	×	×	×	×			×		×		×	×		×	×				2	1	
1913	Niemann				×		×						×		×							2	
,,	Niemann		×		×		×						×		×								
,,	Frank																						
,,	Wolff				×		×						×		×							1	
1912		Schlossmann		×	×			n			×			×		×					1	3	
1913		Schlossmann		×	×																		
1913		Schlossmann-Murschhauser		×	×			n			×			×	×	×						1	The influence of activity
1914		Benedict-Talbot	×	×	×		×	×					×		×	+			1		9	27	
1915		Benedict-Talbot		×	×		×	×					×		×	+				94			
,,	Rubner-Langstein			×	×								×		×				2				
,,		Murlin	×		×	×	×		×				×										
,,		Murlin-Hoobler		×	×	×		×					×	×		×					4	5	
,,		Bailey-Murlin		×	×								×	×		×				6			
1919		Harris--Benedict		×	×																		
,,		Benedict-Talbot	×	×	×			×					×		×	+					62		A biometric study
,,	(Bailey)			×	×																		
,,		Flemming		×	×				×				×	×		+			2			26	
,,		Talbot		×	×			×							×								Standard values
,,		Talbot		×	×								×		×	×						17	
1922	Marine		×	×	×																		
,,	Marine et al.			×	×			×					×		×	+		2		8			High RQ values

1) n = night

App. V. *A schematic summary of the literature (continued)*

Year of publication	Author: Using open method	Author: Using closed method	Description of apparatus	Determination of: O_2	Determination of: CO_2	Determination of: Urinary N_2	Investig: Time: Long	Time: Short: a. m.[1]	Time: Short: p. m.
1923		Talbot et al.	×	×	×				×
,,		Murlin	×	×	×	×			×
1924		Flemming-Hutchisson		×	×				×
,,	Kestner-Knipping			×	×				
,,		Fouet		×	×			×	
1925		Murlin et al.		×	×				
,,		Marsh et al.		×	×				
1926	Kestner		×	×	×				
,,		Levine et al.	×	×	×	×			
1927		Levine et al.	×	×	×	×			×
1928		Levine et al.		×	×	×			×
,,		Wilson et al.		×	×	×			×
,,		Levine et al.		×	×	×			×
,,	Knipping-Rona		×	×	×				
1929	Janet-Bochet		×		×				
,,	Baer			×	×				
1930	Schadow			×	×				
,,	Higgins-Bates		×	×	×				
,,		Wilson et al.		×	×	×			×
1931		Levine et al.		×	×	×			×
,,	Bochet		×	×	×				
,,	Janet-Bochet		×	×	×			×	
,,	Beck			×	×				×
1932	Schadow			×	×			×	
,,		Essig		×	×				×
,,		Essig	×	×	×				×
1933	Mordhorst		×	×					×
,,		Wyatt et al.		×	×				×
,,		Talbot		×	×				×
,,	Schlossmann-Murschhauser			×	×				n
,,	Janet-Bochet		×	×	×			×	
,,	Garot			×	×			×	
1935		Levine e al.		×	×	×	×		
,,		Gordon et al.		×	×	×			×
,,		Gordon et al.		×	×	×			
,,	Räihä		×	×	×				
,,	Lewis et al.		×	×	×				
,,	Newburgh et al.		×	×	×				
1938		Talbot							
1939	Higgins-MacLean		×	×	×				
1940		Gordon et al.		×	×		×		
1941	Clagett-Hathaway		×	×	×				×
1942		Dany et al.	×	×	×	×			×
1943	Benjamin-Weech		×	×	×			×	
1944		Kaijser	×	×	×				
1948		Day		×	×	×			×
1948	Malm		×	×	×				
1951	Malm		×	×	×				

1) n = night

lation from			Number of cases					Comments
			Premature		Full term			
^{2}	RQ[2)]	Urinary N_2	New-born ↓ <1 wk	>1 wk ↓ < year	New-born ↓ <1 wk	> 1 wk ↓ < 1 year	Under-weigt	
						2		
×				1	4			
×					85			Low RQ values
						1		
						1	1	
×						7		High RQ values
+					7			
+			3		17			
+						1		
						1		The influence of food
+			3		9	6		
×						1		
						1		Myxoedema
					1	-		
×						3		
×							2	The influence of temp.
						2		
					×			
×	×					2	1	
							2	
							1	
×						1	3	
								The influence of activity
×							1	
+				1		9	27	
+					94			
				2				
×						4	5	
×					6			
+						62		A biometric study
+				2			26	
×							17	Standard values
+			2		8			
								High RQ values

App. V. *A schematic summary of the literature (continued)*

Year of publication	Author: Using open method	Author: Using closed method	Description of apparatus	Determination of: O_2	Determination of: CO_2	Determination of: Urinary N_2	Investigations conditions: Time: Long	Time: Short: a. m.[1)]	Time: Short: p. m.	Premedication	Food: Fasting	Food: Tea	Food: Small meal	Calculation from: O_2	Calculation from: CO_2	Calculation from: RQ[2)]	Calculation from: Urinary N_2	Number of cases: Premature: New-born ↓ <1 wk	Number of cases: Premature: >1 wk ↓ < year	Number of cases: Full term: New-born ↓ <1 wk	Number of cases: Full term: > 1 wk ↓ < 1 year	Number of cases: Under-weigt	Comments
1923		Talbot et al.	×	×	×				×				×		×	+		4	18				
„		Murlin	×	×	×	×			×				×	×		×							Conclusions
1924		Flemming-Hutchisson		×	×					×			×	×		+		3	2			9	
„	Kestner-Knipping			×	×																		Standard values
„	Fouet			×	×			×			×		×	×		×			12	1	10	7	
1925		Murlin et al.		×	×								×	×		+		1		37			
„		Marsh et al.		×	×								×	×		+		13	2	1			
1926	Kestner		×	×	×																		
„		Levine et al.	×	×	×	×																	
1927		Levine et al.	×	×	×	×			×				×	×		×					2		
1928		Levine et al.		×	×	×			×				×	×		+					4	6	
„		Wilson et al.		×	×	×			×				×	×		×					3	3	The influence of food.
„		Levine et al.		×	×	×			×				×	×		×					4	4	The influence of food.
„	Knipping-Rona		×	×	×																		
1929	Janet-Bochet		×		×																		
„	Baer			×	×					×	×			×		+			2		13		
1930	Schadow			×	×					×		×		×		×				10	40		
„	Higgins-Bates		×	×	×																		
„		Wilson et al.		×	×	×			×				×	×		×					×		The influence of activity.
1931		Levine et al.		×	×	×			×				×	×		+			3		7	4	
„	Bochet		×	×	×																		
„	Janet-Bochet		×	×	×			×			×		×	×		×					42		
„	Beck			×	×				×					×		+					4		The influence of fever.
1932	Schadow			×	×			×		×	×			×		+		2	20		1		
„	Essig			×	×				×					×		×					×		The influence of fever.
„	Essig		×	×	×				×					×					6			9	
1933	Mordhorst		×	×					×	×		×		×					8	2	6	3	
„		Wyatt et al.		×	×				×				×	×		+	×				3	1	The influence of thyroid.
„		Talbot		×	×				×				×		×	×			6			10	Study of twins.
„	Schlossmann-Murschhauser			×	×				n				×	×		+		2	1	7			
„	Janet-Bochet		×	×	×			×			×		×	×		×					80	29	
„	Garot			×	×			×				×		×		+	×				8	6	
1935		Levine e al.		×	×	×	×						×	×		+	×				3		Daily energy requirements
„		Gordon et al.		×	×	×			×				×	×		+	×				1	1	
„		Gordon et al.		×	×	×							×	×		+	×	11	19				
„	Räihä		×	×	×																		
„	Lewis et al.		×	×	×																		
„	Newburgh et al.		×	×	×																		
1938		Talbot																			×	×	Standard values.
1939	Higgins-MacLean		×	×	×																		
1940	Gordon et al.			×	×		×						×						11				Daily energy requirements.
1941	Clagett-Hathaway		×	×	×				×				×	×		×	×				8		Prolonged study.
1942	Dany et al.		×	×	×	×			×				×	×		+	×		10		3		The influence of food.
1943	Benjamin-Weech		×	×	×			×		×			×		×	+					4		Prolonged study.
1944	Kaijser		×	×	×																		
1948	Day			×	×	×			×				×	×		×	×	9	20				
1948	Malm		×	×	×																		
1951	Malm		×	×	×																		

1) n = night